STAIN TECHNOLOGY

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FRANCIS P. GARVAN

In Memoriam

It is a matter of great regret to report the death of Mr. Francis P. Garvan, president of the Chemical Foundation, who succumbed to pneumonia on November 7, 1937, after a few days' illness. Altho trained as a lawyer, not as a scientist, few men in this country have done more than Mr. Garvan for the development of research along chemical and biological lines. His loss will be felt keenly.

The writer's most vivid recollection of Mr. Garvan concerns an interview in his office sometime over twelve years ago, before this journal had come into existence. The subject under discussion was what developments in connection with biological stains might be possible if increased funds were put at the disposal of the Stain Commission. In the course of the interview Mr. Garvan suddenly jumped up from his chair and walked to the side of the room, where he stood a minute with his back toward the others seated near his desk, then wheeled around and asked the question, "Is there any journal at present devoted to stains?" On being informed that there probably was not, he came back with the words, "Well, there will be some day. Why not preëmpt the field?" Thus was STAIN TECHNOLOGY conceived.

This is merely one little illustration of the energy and quick-thinking for which he was noted. In addition to this, his many interests and great versatility have combined to make him one of the outstanding men of the period. It would require pages to describe his numerous interests, many of which have resulted in direct contributions to science. Among them can be mentioned: the advancement in chemical education, resulting in the establishment of a journal on that subject and a chair in chemical education at Johns Hopkins University; the endowment of prizes for college and high school essays in chemistry and for research in special fields of the subject; furnishing financial assistance to chemical journals and the American Journal of Cancer; stimulation of research on dyes; also research in sewage disposal and in the development of chemical by-products of the farm.

A recital of his various lines of interest makes it difficult to believe that his profession was law. Yet, in 1889, two years after graduating from Yale, he received his LL.B. from New York Law School. He

had already distinguished himself as a lawyer before the events began in 1917 which brought him particularly to the country's attention. In that year the entrance of the United States into the World War brought into the possession of the government much confiscated property that had previously been of German ownership. Mr. Garvan at this period was made director of the Bureau of Investigations of the office of Alien Property Custodian, and in 1919 became Alien Property Custodian himself. He appreciated the necessity of developing a chemical industry in the United States to break the monopoly in that field previously held by Germany, which had prevented progress in this country thru patents on its chemical discoveries taken out in America. These patents, however, were now the property of the American government and were held by the Alien Property Custodian. Mr. Garvan saw the chance this offered to develop a chemical industry in the United States.

To dispose of these patents in such a way as to prevent their abuse on the part of selfish interests, he organized the Chemical Foundation as a non-profit organization to purchase them from the government. Later in 1919 he resigned from the offices he held under the government and became president of the newly created Chemical Foundation. He served in this capacity until his death, not only without salary but with the necessity of frequently assisting the Chemical Foundation from his private finances.

There is no need here of discussing the various interests of the Chemical Foundation. It is enough to say that one of these has been the standardization of biological stains. Coöperating with the National Research Council, in 1920, the officers of the Chemical Foundation learned that a committee of the Society of American Bacteriologists had begun investigations to locate reliable American stains. As this seemed to be directly in line with the ideals of Mr. Garvan in establishing the Chemical Foundation, they offered a contribution to help organize this work. The Commission on Standardization of Biological Stains, organized a year or two later, has been supported very liberally by the Foundation, having received in all nearly $100,000 from this source. It must be realized, moreover, that indirectly much of this may have come from personal contributions made by Mr. Garvan.

Without this financial aid the accomplishments of the Commission would not have been possible. Even STAIN TECHNOLOGY, which now yields a profit, would hardly have been undertaken without Mr. Garvan's offer to underwrite it, and its publication could never have been managed but for the clerical help the Commission has been able to command, thanks to the generous support of the Foundation. And it always has to be remembered that this journal was originally the idea of Mr. Garvan himself.—H. J. CONN.

STAINING NERVE FIBERS WITH SILVER IN TISSUE FIXED WITH BOUIN'S FLUID

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ABSTRACT.—**Nerve fibers, in organs fixed with Bouin's fluid, are usually refractive to the Davenport silver technic. The axons, however, can be successfully stained if the sections, on slides, are given a preliminary treatment with concentrated pyridine (1 hour), then a 24-hour bath of ammoniated alcohol (99 cc. 80% alcohol, 1 cc 28% ammonium hydroxide) and an interval in 40% aqueous silver nitrate (6–8 hours) before being immersed in the acidified alcoholic silver solution of Davenport. Following the silvering, reduction and toning of the axons, according to the procedure of Davenport, the surrounding non-nervous tissue elements can be counterstained with a combination of either azocarmine, light green and orange G, or azocarmine, aniline blue and orange G.**

Nerve fibers in organs which are fixed in Bouin's fluid are usually refractive to the Davenport silver methods. Because of the universal use of this fluid for fixing tissues for routine study, it is believed that a technic which adequately displays the axons and yet leaves the section as well as the block available for other stains will be of interest to students of microtechnic. For these reasons the writer submits a silver technic for routine use with sections of paraffin embedded material. The method gives a satisfactory staining of axons in a variety of organs (Figs. 1–4) but is not recommended for quantitative studies on peripheral nerves which contain minute fibers. For the latter, the technic gives better results if the nerves are fixed in 10% formalin to which 1.0% monochloracetic acid has been added or in acidified alcoholic picro-formalin (picric acid 0.2 g., abs. alcohol 99 c.c., Mercks blue label formaldehyde 1 c.c., monochloracetic acid 1 g.).

PROCEDURE

The slides, with sections (5–20 μ) affixed, are passed thru xylene and absolute alcohol.

From absolute alcohol they are placed in concentrated pyridine for 1 hour, rinsed in absolute alcohol, passed rapidly thru ether-alcohol and placed in a 2–4% ether-alcohol solution of nitrocellulose for 1–5 minutes, after which they are removed and given a thin coating of nitrocellulose.[1]

[1]Foley, J. O. 1936. Suggested counterstains for Davenport reduced silver preparations of peripheral nerves. Stain Techn., **11**, 3–8.

PLATE I, EXPLANATION OF FIGURES

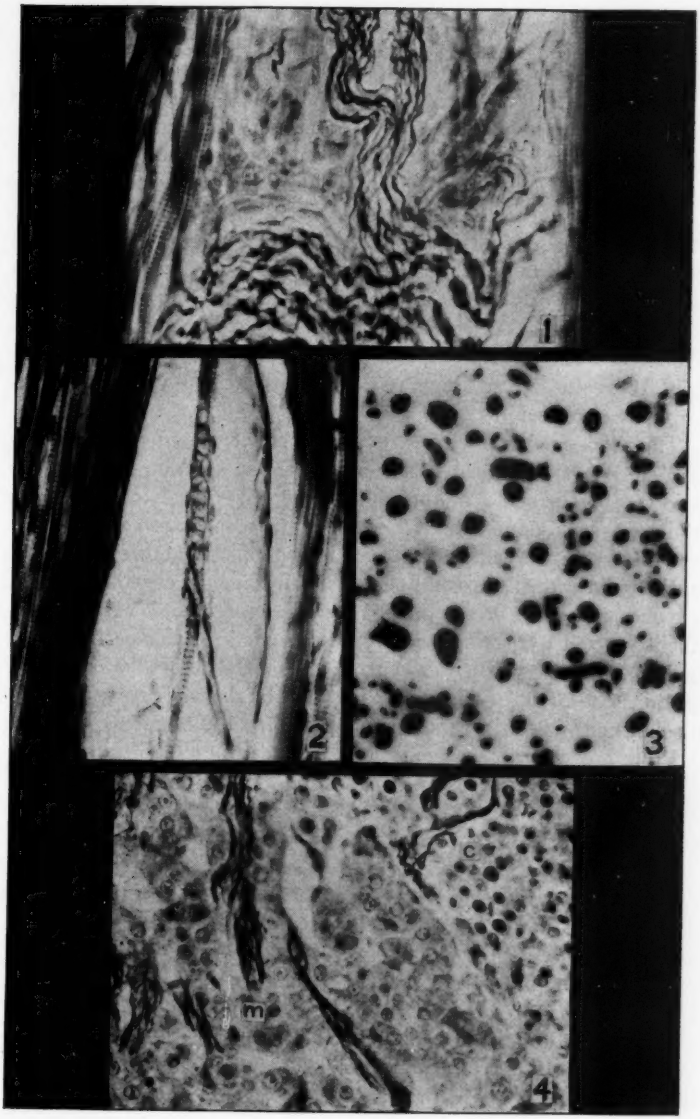
Photomicrographs of tissues in which the axons have been stained with silver after fixation in Bouin's fluid.

1. A 20 μ longitudinal section thru one of the strap muscles of the guinea pig ($\times$ 450 diameters). Note the two sizes of nerve fibers.

2. A 20 μ longitudinal section thru one of the strap muscles of the guinea pig ($\times$ 450 diameters). Attention is directed to the muscle spindle in the center of the photograph.

3. A 7 μ cross section of the vagus nerve of the cat above the nodose ganglion ($\times$ 2000 diameters). Observe the range in size of the axons.

4. A 20 μ section thru the adrenal gland of the guinea pig ($\times$ 450 diameters). Note the bundles of axons in the cortex (c) and medulla (m). Likewise, attention is directed to the histological detail shown in the non-nervous structures in Figs. 1, 2 and 4.


1
2
3
m
4

After coating, the slides are placed in a solution of ammoniated alcohol (99 cc. 80% alcohol, 1 cc. 28% ammonium hydroxide) for 12–24 hours. From ammoniated alcohol they are rinsed very briefly (3–5 seconds) in 80% and transferred to a 40% aqueous solution of silver nitrate (in oven at 37.5° C.) 6–8 hours. The use of 40% silver nitrate is in line with the finding of Rogers[2] on the value of using concentrated solutions of silver for staining nerve fibers in sections.

From the aqueous silver bath the slides are placed directly into Davenport's[3] acidified alcoholic solution of silver nitrate (dissolve 20 g. silver nitrate in 20 cc. dist. water; add 0.1–0.5 cc. normal nitric acid and make up to 100 cc. with 95% alcohol) at room temperature for 16–24 hours. The amount of nitric acid which must be added to the silver bath to give the best results can only be determined by trial, the values given above represent approximately the upper and lower limits of acidity.

The slides are removed from alcoholic silver, rinsed very briefly in 95% alcohol and placed in a 5–8% alcoholic solution of pyrogallic acid (pyrogallic acid 8 g., formaldehyde 5 cc., 95% alcohol 95 cc.) to which has been added about 1 cc. of dilute yellow Karo syrup (Karo and distilled water, equal parts). The sections are allowed to remain in the reducer until the smallest axons are stained a chocolate brown, the endpoint being determined by frequent microscopic examination. Eight percent pyrogallic acid is recommended for thin sections (5 μ or less), whereas weaker concentrations should be used with thicker sections (10–20 μ). Following reduction, the slides are rinsed in 95% alcohol and placed in running tap water for 1–3 minutes.

From tap water the preparations are toned in a 0.2% aqueous solution of neutral gold chloride for 10 minutes, after which they are washed in running tap water and placed in a 5.0% aqueous solution of sodium thiosulphate for 3–5 minutes.

After thoroly washing in tap water the coating of nitrocellulose is removed by passing the slides thru 75, 95, and 100% alcohol and two changes of acetone, agitating in the latter to prevent precipitate from settling on the sections.

From acetone the preparations are run thru absolute alcohol and xylene and mounted in dammar.

If desired, the sections may be counterstained with azocarmine followed by light-green-orange-G, or azocarmine followed by aniline-blue-orange-G. (See footnote 1.)

[2]Rogers, W. M. 1931. New silver methods for paraffin sections. Anat. Record, 49, 81–7.

[3]Davenport, H. A. 1930. Staining nerve fibers in mounted sections with alcoholic silver nitrate solution. Arch. Neur. & Psych., 24, 690–5.

A COMPARATIVE STUDY OF SOME DEHYDRATION AND CLEARING AGENTS[1]

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ABSTRACT.—**An experiment to determine the advantages of dioxan, iso-butyl alcohol, tertiary butyl alcohol, and ethyl alcohol as dehydrants and chloroform, toluol, xylene, benzol, methyl benzoate, methyl salicylate, and acetone as clearers is described. Materials fixed in Bouin's fluid, Zenker formol, and 10% neutral formalin were dehydrated, embedded, sectioned, and stained. Bouin's fluid produces less hardening, shrinkage and distortion than the other fixatives employed. Slow dioxan is the best method of dehydration. All the picric acid need not be removed from tissues to be embedded in paraffin. Tissue blocks not more than 4 mm. thick may be dehydrated and impregnated with paraffin by slow dioxan in 13 hours, fast dioxan in 10 hours, iso-butyl alcohol and tertiary butyl alcohol in 14 hours, and ethyl alcohol-chloroform in 17 hours without incurring any distortion due to rapidity of dehydration and infiltration.**

Recently many workers have been concerned with the problem of finding reagents which would produce a minimum of distortion, shrinkage, and hardening in the paraffin method of embedding animal tissues. Their investigations have followed three lines: that of finding reagents to take the place of ethyl alcohol as a dehydrant, the search for an improvement upon xylene as a clearer following ethyl alcohol, and the use of different types of fixatives.

The purpose of this study has been to coördinate these three lines of investigation and if possible to arrive at some conclusion as to which reagent or reagents produce the least amount of shrinkage, hardening, and distortion. Dioxan, iso-butyl alcohol, tertiary butyl alcohol, and ethyl alcohol were used as dehydrants. Chloroform, toluol, xylene, methyl benzoate, benzol, methyl salicylate, and acetone were the clearers used to follow ethyl alcohol. Fixatives used were 10% neutral formalin, Zenker formol, and Bouin's fluid, chosen because they represent the three most widely used reagents.

Shrinkage has been attributed to improper fixation, exposure to air during the process of fixation and dehydration, and the action of

[1]Contribution from the Zoology Laboratory, Oklahoma Agricultural and Mechanical College; prepared under G. A. Moore. For helpful suggestions the writer is indebted to Dr. J. E. Lynch, Department of Fisheries, University of Washington.

reagents. Hardening has been attributed to the type of fixative used, the influence of hot paraffin, and the action of reagents. Distortion has been largely attributed to changing tissues from liquids of varying densities and temperatures, and to pronounced shrinkage of particular organ elements such as connective tissue. In this study an effort was made to remove any possibility of differences in results due to factors other than the characteristic effects of the dehydrant, clearer, and fixative.

Four tissues were used—thyroid gland, intestine, liver, and skeletal muscle. These were taken from the same animal and fixed at the same time. From each tissue 5 blocks were fixed in Zenker formol, 5 in formalin, and 11 in Bouin's fluid. Small adjacent pieces, 4 mm. × 4 mm. × 1 cm. were fixed (24 hours) to insure best results. Blocks fixed in Bouin's fluid were transferred directly from the fixative to the first step of dehydration. Those in formalin were washed in running tap water for 10 hours, those in Zenker formol for 15 hours, before starting dehydration.

It will be noted that except in the case of "fast dioxan," dehydration was effected gradually in order to eliminate differences due to variations in the density of the reagents. This has not been taken into account in some previous studies. "Slow dioxan", fast dioxan, iso-butyl alcohol, tertiary butyl alcohol, and ethyl alcohol, followed by the clearers already mentioned, were used. Because of the relative immiscibility of iso-butyl alcohol with water, it was found advisable to extract most of the water with ethyl alcohol. The remainder of the water and the ethyl alcohol were removed with iso-butyl alcohol, with which ethyl alcohol is quite miscible.

The paraffin mixtures were kept at 54–56 degrees C. during the process of embedding. The embedding medium was a 3.5% mixture of yellow beeswax in soft paraffin.

The detailed procedures for dehydration and clearing are as follows:

Iso-butyl alcohol: 30% ethyl alcohol, 2 hr.; 50% ethyl alcohol, 2 hr.; 70% ethyl alcohol, 2 hr.; 95% iso-butyl alcohol, 3 hr.; iso-butyl alcohol, 1 hr.; 50% iso-butyl alcohol, 50% paraffin, 1 hr.; hard paraffin, 2 hr.; hard paraffin, 1 hr.; embed.

Tertiary butyl alcohol: 30% tertiary butyl alcohol, 2 hr.; 50% tertiary butyl alcohol, 2 hr.; 70% tertiary butyl alcohol, 2 hr.; 95% tertiary butyl alcohol, 3 hr.; tertiary butyl alcohol, 1 hr.; 50% tertiary butyl alcohol, 50% paraffin, 1 hr.; hard paraffin, 2 hr.; hard paraffin, 1 hr.; embed.

Slow dioxan: 33% dioxan, 2 hr.; 67% dioxan, 2 hr.; dioxan, 4 hr.;

33% paraffin, 67% dioxan, 1 hr.; 67% paraffin, 33% dioxan, 1 hr.; hard paraffin, 2 hr.; hard paraffin, 1 hr.; embed.

Fast dioxan: Dioxan, 2 hr.; dioxan, 4 hr.; 50% paraffin, 50% dioxan, 1 hr.; hard paraffin, 2 hr.; hard paraffin, 1 hr.; embed.

Ethyl alcohol-chloroform: 30% ethyl alcohol, 2 hr.; 50% ethyl alcohol, 2 hr.; 70% ethyl alcohol, 2 hr.; 95% ethyl alcohol, 3 hr.; ethyl alcohol, 1 hr.; 50% ethyl alcohol, 50% chloroform, 1 hr.; chloroform, 1 hr.; 67% chloroform, 33% paraffin, 1 hr.; 33% chloroform, 67% paraffin, 1 hr.; hard paraffin, 2 hr.; hard paraffin, 1 hr.; embed.

Ethyl alcohol-toluol,-xylene,-benzol,-methyl benzoate,-methyl salicylate, and -acetone: the procedures for these clearers are the same as that of ethyl alcohol-chloroform except that a mixture of 50% clearer and 50% paraffin is used for 1 hr. instead of the two chloroform-paraffin baths for 2 hr.

The blocks were cut at 10 μ, the room temperature ranging from 22.5 to 31° C. The sections were flattened by placing the ribbon on water at 40° C. This permitted perfect flattening of the sections without stretching or distortion. The sections were floated on slides lightly coated with Mayer's albumen. The slides were dried, decerated in toluol, passed down to absolute alcohol after which they were immersed in 0.5% pyroxylin, and dried in air until a slight film appeared on the pyroxylin. The slides were plunged into 95% alcohol and downgraded to Harris's hematoxylin in which they were stained. After upgrading they were counterstained with triosin in 95% alcohol, after which they were soaked in absolute alcohol until the pyroxylin was removed. The slides were then immersed in 1% pyroxylin[2] which was hardened in chloroform, after which they were cleared in toluol. A comparison was made of two sets of slides which differed only with regard to the second coat of pyroxylin. The slides without the second coating showed much more shrinkage, particularly of the connective tissue layers. This was due, no doubt, to the shrinking effect of the toluol in clearing.

Tables 1 and 2 give the results of the procedures mentioned above. Due to the fact that there was no objective means of measuring the qualities of the blocks and slides, they were examined independently by two investigators and their joint findings compiled. In regard to impregnation it was found that for tissue blocks of this size, three hours in paraffin were sufficient to bring about complete infiltration (for results see tables—column 2 under impregnation). In order to investigate the reagents in regard to impregnation the experiment was repeated with the same procedure except that the tissues were left in

[2]Equivalent of celloidin as suggested by Galigher (1934).

TABLE 1. TABLE SHOWING THE EFFECT OF DEHYDRATING AND CLEARING REAGENTS ON THYROID GLAND AND SKELETAL MUSCLE. SD, SLOW DIOXAN; FD, FAST DIOXAN; IA, ISO-BUTYL ALCOHOL; TA, TERTIARY BUTYL ALCOHOL; EA, ETHYL ALCOHOL; CHL, CHLOROFORM; TOL, TOLUOL; BEN, BENZOL; M BEN, METHYL BENZOATE; M SAL, METHYL SALICYLATE; ACE, ACETONE; SL, SLIGHTLY

TISSUE	FIXATIVE	DEHYDRATION	CLEARER	IMPREGNATION				SECTIONS				MICRO-EXAM					
				1		2						Shrinkage			Cracking		
				Com	Inc	Com	Inc	Soft	Medium	Hard	Shatters	None	Medium	Bad	None	Medium	Bad
THYROID	ZENKER	SD		+		+		+				+				+	
		FD		+		+		+					+			+	
		IA		+		+		+				+			+		
		TA				+			+				+			+	
		EA	CHL	+		+		+					+		+		
	FORMALIN	SD			+	+			+				+		+		
		FD		+		+		+					+		+		
		IA		+		+		+					+			+	
		TA				+		+					+		+		
		EA	CHL	+		+		+						+	+		
	BOUIN	SD		+		+			+			+			+		
		FD			+	+		+				+			+		
		IA		+		+		+					+			+	
		TA				+		+					+		+		
		EA	CHL	+		+		+					+			+	
		EA	TOL		+	+		+					+				+
		EA	XYLOL	+		+		+					+			+	
		EA	BEN	+		+		+					+			+	
		EA	M BEN		+	+		+						+		+	
		EA	M SAL		+	+		+					+			+	
		EA	ACE		+	+		+					+			+	
SKELETAL-MUSCLE	ZENKER	SD			+	+			+		+			+			+
		FD		+		+				+	+			+		+	
		IA		+		+				+	+			+			+
		TA				+				+				+			+
		EA	CHL	+		+				+				+		+	
	FORMALIN	SD		+		+		+					+			+	
		FD		+		+		+					+			+	
		IA			+	+				+	+			+			+
		TA				+				+	+			+		+	
		EA	CHL	+		+				+	+			+		+	
	BOUIN	SD		+		+		+				+			+		
		FD		+		+				+		+			+		
		IA		+		+			+		SL		+		+		
		TA				+			+		SL		+		+		
		EA	CHL	+		+		+					+		+		
		EA	TOL	+		+				+			+		+		
		EA	XYLOL	+		+			+		+		+		+		
		EA	BEN	+		+			+		SL	+			+		
		EA	M BEN	+		+				+	+		+		+		
		EA	M SAL	+		+			+		+		+		+		
		EA	ACE	+		+			+		SL		+			+	

TABLE 2. TABLE SHOWING THE EFFECT OF DEHYDRATING AND CLEARING REAGENTS ON LIVER AND INTESTINE. SD, SLOW DIOXAN; FD, FAST DIOXAN; IA, ISO-BUTYL ALCOHOL; TA, TERTIARY BUTYL ALCOHOL; EA, ETHYL ALCOHOL; CHL, CHLOROFORM; TOL, TOLUOL; BEN, BENZOL; M BEN, METHYL BENZOATE; M SAL, METHYL SALICYLATE; ACE, ACETONE; SL, SLIGHTLY

TISSUE	FIXATIVE	DEHYDRATION	CLEARER	IMPREGNATION				SECTIONS				MICRO-EXAM					
				1		2						Shrinkage			Cracking		
				Com	Inc	Com	Inc	Soft	Medium	Hard	Shatters	None	Medium	Bad	None	Medium	Bad
LIVER	ZENKER	SD		+		+		+									
		FD		+		+		+									
		IA			+	+		+									
		TA				+		+									
		EA	CHL	+		+			+								
	FORMALIN	SD		+		+		+									
		FD		+		+		+									
		IA		+		+		+									
		TA				+		+									
		EA	CHL	+		+		+									
	BOUIN	SD		+		+		+									
		FD		+		+		+									
		IA			+	+			+								
		TA				+		+									
		EA	CHL	+		+		+									
		EA	TOL	+		+		+									
		EA	XYLOL	+		+		+									
		EA	BEN	+		+		+									
		EA	M BEN	+		+		+									
		EA	M SAL	+		+		+									
		EA	ACE	+		+		+									
INTESTINE	ZENKER	SD		+		+		+				+			+		
		FD		+		+		+					+		+		
		IA			+	+		+					+			SL	
		TA				+			+					+		+	
		EA	CHL		+	+		+					+		+		
	FORMALIN	SD		+		+			+					+	+		
		FD		+		+				+			+		+		
		IA		+		+				+				+		SL	
		TA				+			+				+			SL	
		EA	CHL	+		+			+				+			+	
	BOUIN	SD		+		+		+				+				+	
		FD		+		+			+			+				+	
		IA		+		+		+					+				+
		TA				+			+				+			+	
		EA	CHL	+		+		+					+				+
		EA	TOL	+		+		+					+				+
		EA	XYLOL	+		+		+					+			+	
		EA	BEN	+		+		+				+					+
		EA	M BEN	+		+		+				+					+
		EA	M SAL	+		+		+				+				+	
		EA	ACE	+		+		+					+				+

hard paraffin only two hours. The results of this experiment are tabulated in column 1 under impregnation. Iso-butyl alcohol shows the greatest difficulty in impregnation, probably due to its limited miscibility with paraffin and water. Dioxan showed a tendency to require more time in infiltration than all other dehydrants except iso-butyl alcohol. Slow dioxan after fixation in Zenker formol and in formalin produced much softer blocks than all other methods. After fixation with Bouin's fluid, ethyl alcohol-chloroform, ethyl alcohol-xylene, ethyl alcohol-benzol, ethyl alcohol-methyl salicylate, and ethyl alcohol-acetone produced blocks equally soft, but with some shattering. Of all the clearers tried chloroform produced the softest blocks and toluol and methyl benzoate the hardest blocks. Xylene, benzol, methyl salicylate, and acetone were of equal value. Slow dioxan and fast dioxan seemed to be equally effective in eliminating shrinkage and excelled all other methods. The most shrinkage was produced by iso-butyl alcohol and tertiary butyl alcohol. Ethyl alcohol-chloroform, ethyl alcohol-toluol, ethyl alcohol-methyl benzoate, ethyl alcohol-acetone, ethyl alcohol-benzol, and ethyl alcohol-methyl salicylate were of equal value. There was less cracking with slow dioxan, fast dioxan, and tertiary butyl alcohol than with other methods.

In regard to fixation, Bouin's fluid produced by far the best results, with softer blocks and less shrinkage. There was little shattering with even the most difficult tissues. To determine the effects of the presence of picric acid in tissues an experiment was conducted with duplicate tissue blocks and with the same procedure, except that in one all of the picric acid was washed out with lithium carbonate in 70% alcohol. There were no differences in cutting consistency between blocks prepared in this manner and those in which much of the picric acid was left.

Zenker formol produced the hardest blocks of the three fixatives used. The hardening took place mostly during fixation. In regard to shattering Zenker formal and 10% formalin were of approximately the same value, quite inferior to Bouin's fluid. Formalin produced initial softness but much hardening took place during dehydration and embedding. Formalin fixation was attended in all cases by a disintegration, and an alternation of the staining reaction, of thyroid colloid, and produced considerable shrinkage. It was the least satisfactory of the fixatives used.

The best complete method was found to be Bouin's fixation with dehydration by the slow dioxan method. For student use, however, this has the objection of expense coupled with some danger from inhalation of the fumes. Perhaps the most satisfactory method for student work is Bouin's fixation followed by ethyl alcohol-chloro-

form. This method produces soft blocks with little shrinkage or cracking.

Conclusions

(1) Bouin's fixative produces less hardening, shrinkage, and distortion than the other fixatives employed.

(2) Slow dioxan is the best method of dehydration.

(3) Picric acid need not all be removed from tissues to be embedded in paraffin.

(4) Tissue blocks 4 mm. thick may be dehydrated and impregnated with paraffin by slow dioxan in 13 hr., fast dioxan in 10 hr., and ethyl alcohol-chloroform in 17 hr., without incurring distortion due to rapidity of dehydration and infiltration.

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DIFFERENTIATION OF TWO CLASSES OF ACIDOPHILES IN THE ANTERIOR PITUITARY OF THE FEMALE RABBIT AND CAT

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ABSTRACT.—**A differential stain for the anterior pituitary of mammals, based directly on Heidenhain's 'azan' modification of Mallory's connective tissue stain has been devised. Tissue is fixed for 24 hours in a saturated solution of corrosive sublimate in physiological saline (90 parts) and formalin (10 parts) and washed directly in 70% alcohol for 48 hours. Sections are treated on the slide with a 3% solution of potassium bichromate for 12 hours. Two classes of acidophiles are demonstrated: one which stains selectively with azocarmine; and the ordinary acidophile which stains with orange G. The special acidophile has been demonstrated in the female rabbit and cat but has not been found in the mouse or rat.**

At the present time the number of active principles obtained from the anterior pituitary greatly exceeds the number of cells that can be differentiated by any of the several selective stains in general use (Cleveland and Wolfe, 1932, Severinghaus, 1932). Only three main categories of cells, designated by various letters and numbers, but commonly referred to as acidophiles, basophiles and chromophobes are generally recognized. The two chromophilic types may exhibit great variation in cell size, size of specific granules and degree of granulation and degranulation, but no selective staining methods have been developed which will sharply differentiate them tinctorially into subgroups.

In the course of an extensive study of the cytological changes accompanying sexual activity in the female rabbit (Friedgood and Dawson, 1937) a method was developed which separated the acidophiles into two distinct classes. The staining reaction was first obtained from material which had been fixed in 10% formalin and stained by a slight modification of Heidenhain's 'azan' modification of Mallory's triple connective tissue stain. In these preparations the acidophiles were stained deep red and brilliant orange. Since the formalin alone did not give a good fixation of basophiles and chromophobes, the fixing fluid was modified by using a saturated solution of

corrosive sublimate in physiological saline and formalin, in the proportions of 9:1. This fluid gave excellent and uniform fixation of entire rabbit and cat pituitaries and did not seriously affect the selective staining obtained when formalin only was used. The procedure is as follows:

1. Fix in a sat. solution of mercuric chloride in physiological salt solution (90 parts) and formalin (10 parts) for 24 hours. Wash directly in 70% alcohol for 24–48 hours, dehydrate and embed. Cut 3–4 μ thick.
2. Post-chromation on the slide for 12 hours in 3% solution of potassium bichromate.
3. Stain sections in a 0.2% solution of azocarmine[1] (Grübler) in 1% acetic acid at 55° C. for 1 hour. Allow to cool.
4. Rinse in distilled water.
5. Differentiate under the microscope with 0.1% anilin oil in 95% alcohol until the carmine cells alone remain a deep red. If the differentiation is slow a few drops of distilled water may be added to the alcohol. Differentiation may be controlled by rinsing with acid alcohol.
6. When differentiation is complete rinse with acid alcohol (95% alcohol with 1% acetic acid).
7. Rinse in distilled water.
8. Transfer to 5% phosphotungstic acid for 2 hours.
9. Counterstain in orange G with methyl blue or anilin blue[2] for 12–36 hours depending on the freshness of the stain:

Orange G	2.0 g.
Methyl blue or anilin blue w. s.	0.5 g.
Acetic acid	8.0 cc.
Distilled water	100.0 cc.

10. Rinse with distilled water and examine under the microscope. If insufficiently stained replace in staining solution. If staining is satisfactory, pass directly to 95% alcohol, complete dehydration, clear and mount in balsam.

The differential staining of the acidophiles is also correlated with differences in their granulation. In the cells taking the orange G the granulation appears very fine or almost homogenous, whereas the cells which take the carmine selectively and intensively are characterized by the relative coarseness and refractivity of the granules, which is strikingly demonstrated by oblique illumination. The size and num-

[1]The 0.2 solution of azocarmine is made with boiling distilled water, filtered on cooling and acidified to 1% with acetic acid. This contains very fine needle crystals, but at the staining temperature they are in solution.

[2]Great difficulty has been encountered in obtaining uniform lots of either anilin blue or methyl blue. Grübler's anilin blue has been found most satisfactory, but dyes from other sources have not been given extended trials. National Aniline orange G has given very satisfactory results.

ber of the cells stained with carmine varies with the physiological state of the animal and the size of the granules is somewhat variable. It appears possible that there may be two classes of cells which react with carmine.

If the procedure has been successful the new type of granular cell will be deep red, the ordinary acidophiles orange, the chromophobes light pink to colorless, the basophiles deep blue to light blue, the specific cells of the intermedia deep blue to light blue, and the chief cells of the tuberalis light pink to colorless with the colloid of the vesicles blue.

The great importance of a rigorous standardization of a staining method, if uniform results are to be obtained, has been emphasized by all students of the cytology of the pituitary. The reaction of the several classes of cells to acid and basic dyes is not absolute but relative, and in some instances a cell which has been stained with the one acid dye may subsequently be stained intensely when exposed to a second acid dye.

This is strikingly illustrated when the three dyes of the present method are used separately or with one in excess of the proper proportion. After staining with the azocarmine and before differentiation, all the acidophiles and the dark blue basophiles are stained red. The special carmine cells are dark red, the ordinary acidophiles are light red and the deep blue basophiles of the pars distalis and pars intermedia are a brilliant light red. After differentiation and subsequent staining with the orange G and anilin blue, the ordinary acidophiles are orange and the carmine in the basophiles is completely replaced by the anilin blue.

If the azocarmine is omitted and the tissue stained only with the orange G and anilin blue all the acidophiles are orange. Again if the entire procedure is followed and the proportion of anilin blue is slightly higher than usual, the cells that react with the carmine are reddish orange, the ordinary acidophiles are brown (orange plus blue) and the chromophobes are light blue. With more anilin blue all the cells which normally react with the azocarmine and orange G and the chromophobes may be stained varying intensities of blue, with the orange G and azocarmine completely displaced, or masked. Some of the excess blue may be removed by prolonged treatment with 95% alcohol.

It has been difficult to establish a definite staining routine which will yield uniformly successful preparations. This is due to two factors. The first difficulty is encountered in the differentiation of the azocarmine. Apparently there is considerable variation in the affinity

of the special cell for the carmine. When the cells are large, numerous, and clustered, the differentiation can be readily controlled under the microscope. When they are small and scattered, the endpoint of differentiation is not recognized so readily. The second difficulty comes in the staining with the mixture of orange G and anilin blue. The two dyes do not form a stable mixture and when left standing in the jars an excess of blue tends to accumulate at the top and a staining gradient results. Furthermore when several runs are made with the same mixture of dyes the blue is exhausted more rapidly than the orange G and the length of time required to obtain optimum differentiation is increased. Some of these difficulties may be offset by shaking the mixture vigorously before each staining and by adding a little fresh dye after each batch of slides has been run thru. Even with these precautions uniform staining intensity is not always obtained. In staining with azocarmine care should also be taken to keep the temperature at 55° C. Higher temperatures appear to fix the carmine in the tissue and make it refractory to subsequent differential extraction.

Little can be said about the method of fixation. A fixing fluid with potassium bichromate appears to mask the differential staining of the acidophiles, apparently by increasing the affinity of the granules of the ordinary acidophiles for the azocarmine. This method does not fix the mitochondria so that they cannot be regarded as complicating the granular patterns of the acidophiles. Moreover, preparations fixed in Helly-osmic or Champy and stained with Volkonsky's (1928) modification of Altmann's method gives an excellent differentiation between the specific granules and the mitochondria. In general the mitochondria appear as fine filaments and are not widely scattered thru the cell but are rather sharply restricted to the region of the macula, negative image of the Golgi apparatus.

The foregoing description has been based almost entirely on observations made during the past year on the pituitary of the female rabbit. The special granular cell is characteristic of the sexually mature animal and undergoes striking changes in number and appearance during the reproductive cycle. It is absent or very rarely present in the male. More recently a somewhat similar cell, reacting specifically with azocarmine, has been demonstrated in the female cat. No observations have been made on the pituitary of males. Attempts to demonstrate a similar cell in the female mouse or rat have as yet been unsuccessful.

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A NEW HOME-MADE EMBEDDING PLATE

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Economy and convenience are factors desirable in any laboratory, of which we are reminded by the many helpful suggestions on home-made equipment. Several such pieces of equipment have been in use for some time in the morphological and cytological laboratory of the Division of Pomology of the University of California and have proved satisfactory. It is hoped that the mention here of two of these will be of interest to others and perhaps of value. The illustrations are more descriptive than words hence only a few remarks on each piece of equipment are necessary.

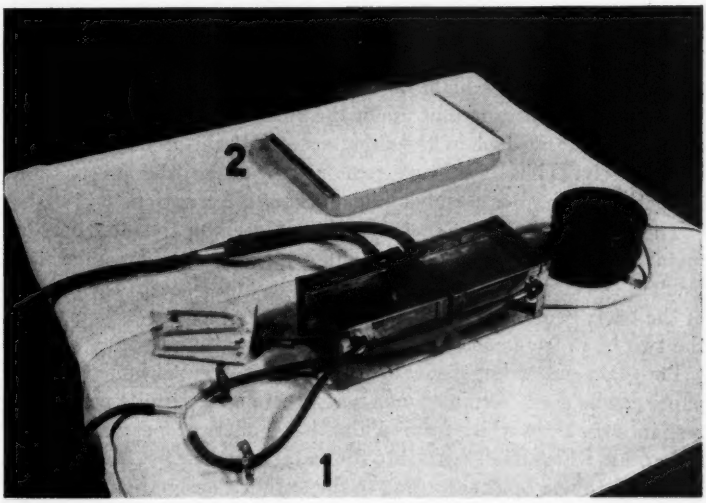

FIGURE A. (1) Embedding plate. The small water inlets are in front; the larger water outlets at the back of the trays. The rheostat is shown at the extreme right; the small heating unit at the extreme left, between the inlet and outlet hose.
(2) Cooling plate for paraffin ribbon.

EMBEDDING PLATE

The plate (Fig. A 1) was designed to facilitate the embedding of more than a few specimens. With the conventional plate it is necessary to move the embedding dish during the process, which usually

deranges the specimens in the paraffin. The entire process, at times, is rather exasperating and unsatisfactory.

This plate eliminates the necessity of moving the embedding dish for cooling by the presence of circulating water in the trays, which rapidly cools the paraffin after the specimens are arranged. The dish can then be lifted out and placed in ice water without the slightest danger of the specimens moving. Water is brought from the faucet into the tray on one side, and let out on the other; the large outlet not permitting the water to rise too rapidly around the embedding dish. Usually by the time it does (according to the amount coming in), the paraffin has solidified enough to support the water. Heat for keeping the paraffin melted during the embedding is provided by the small heating unit that slides from one tray to the other on a track. The rheostat is very convenient for more correctly regulating the amount of heat necessary in embedding, but is not used when the plate is first being heated preparatory to the process. Two trays are not absolutely necessary; but since the introduction of circulating water cools the tray, it requires a little time to reheat for a second embedding. By shoving the heating unit under the second tray when the embedding is about finished in the first, a second embedding can begin immediately. Thus by shifting from one tray to the other it is possible to continue a number of embeddings without interruption.

The rubber hose on the plate may be replaced for the most part by metal tubing, thus reducing the bulkiness of the entire apparatus. Each of the copper trays is 5″ long, 4″ wide, and 1″ deep; the bottom of the trays being 2″ above the brass base. A space of ⅛″ separates the two trays from each other.

Porcelain embedding dishes are most satisfactory; altho parchment paper can be used. Ordinary non-wax proof paper permits paraffin to seep into the trays which forms a film that hinders proper water circulation, and which finally gets into the water outlets.

The plate was built according to the author's design by Mr. Ralph Miller of the Division of Pomology.

Cooling Plate for Paraffin Ribbon

The adhering of paraffin ribbons, in hot weather, to the paper on which they lie can be eliminated by the use of a large tray filled with ice and water (Fig. A 2), which keeps the glass plate over the tray cold and, likewise, the paper containing the ribbons. The latter always remain crisp and very easy to handle.

APPARATUS AND METHODS FOR MICRO-INCINERATION

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ABSTRACT.—**In this article micro-incineration and the necessary apparatus is considered. This in itself is but the first step in determining the mineral content of cells, tissues and organs; but it is a very necessary step as by it one can compare the position and different amounts of ash in individual cells, and in different parts of tissues and organs.**

In 1833 Raspail, founder of chemical microscopy, showed that the original appearance of even the finest detail is represented in the ashes.

The present paper describes the electric furnace originated by the histologist Policard, explains the method of preparation and sectioning objects for incineration, and finally the process itself. The microscopic outfit needed for the study is also described. This consists of an ordinary microscope with an Abbé or aplanatic substage condenser with central dark-stops, of 10, 15 and 20 mm., for the different objectives. These range from 16 mm. to oil immersions, the powers above 16 mm. having iris diaphragms to reduce the aperture a suitable amount.

It is further shown that there is great advantage in having some sections of an object incinerated and neighboring sections stained and mounted for comparison. The incinerated preparations are to be studied with the dark-field and the stained ones with the bright-field microscope, the two instruments close together so that the observer can look from one to the other.

Finally, there is given the method of calibrating the electric furnace so that one may know the temperature at each step in the incineration. There is also a short bibliography of some important publications with brief annotations as to their special significance.

INTRODUCTION AND HISTORICAL SUMMARY

For time out of mind mankind has known in a general way that bones and teeth seem to be made up chiefly of earthy matter. Even primitive man found that in his open fire they did not burn up but retained their original shape, altho they became brittle. Much later, after some chemical knowledge had been gained, it was found that if

bones were put in an acid they appeared unchanged, but they lost their rigidity and could be bent easily.

As knowledge advanced still further it was not difficult to demonstrate that there is considerable inorganic material in even the softest tissues, but whether there is such an intimate connection of mineral and organic matter as with the bones, so that a veritable skeleton in the soft tissues is shown by burning away the organic matter, had to wait a long time for an answer. Finally a French chemist, Raspail (1833), got on the track by spreading a shred of plant epidermis on a glass slip. This was then raised gradually to a red heat, allowed to

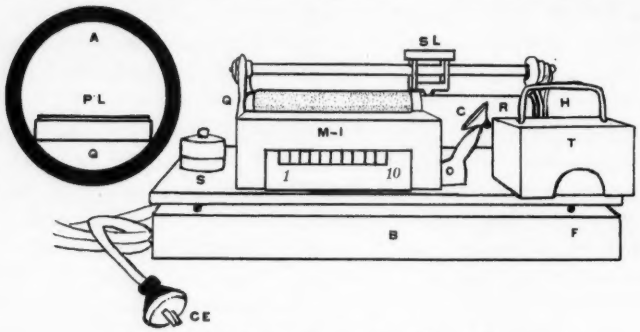


FIG. 1. SCOTT MICRO-INCINERATOR; FRONT VIEW

A End view of the quartz tube in which the sections are incinerated. It is surrounded by a heating coil.
Q Quartz plate for supporting the slide of sections to be incinerated. The platinum plate (PL) shown on its upper face is to prevent slides from sticking. For the Corex and Pyrex slides this expensive part may be omitted.
B Base board of the incinerator; it fits the laboratory lockers.
C Cap or lid to cover one end of the quartz tube.
C E Cap for connecting with the electric circuit.
F Rubber support between the insulating plate and the base.
H Handle of the insulating cover of the quartz tube.
M-I Insulating block containing the quartz tube.
Q Quartz tube; it is surrounded by a heating coil and is embedded in insulating material.
R Variable rheostat with its rider (SL).
SL Rider on the adjustable rheostat to vary the resistance.
T Top of the insulating material covering the quartz tube. It is shown lifted off to hasten the cooling of the quartz tube after an incineration is completed.
1–10 Scale of 10 centimeters to show the amount of reduction.

cool, and then examined under the microscope. The outlines of the cells were clearly indicated by the mineral matter.[1] (See also Liesegang (1910).

[1]The following is a verbatim quotation from p. 529 of Raspail's work, (1833): "On prend une lanière d'épiderme dont les réticulations cellulaires soient bien distinctes, comme sur la figure 7 de la pl. 3, et dont on a préalablement enlevé tous les sels incrustés, au moyen de l'acide hydrochlorique étendu et de lavages répétés. On l'étend

Nearly 100 years later another French scientist, Professor Policard (1923–1932, 1930) of the faculty of medicine in Lyon, while making studies in histo-chemistry, invented a small electric furnace (Figs. 1 & 2) by which any thin section of organic material could be incinerated so as to show its mineral matter practically as it was in the tissue. This electric furnace was regulated by a rheostat (Figs. 1 & 2) so that the heat could be increased gradually to about 600° C.

In such a furnace, not only thin objects like plant epidermis but paraffin sections of any soft tissue can be incinerated to show the mineral matter in place after all the organic matter has been burned away (Fig. 9 B).

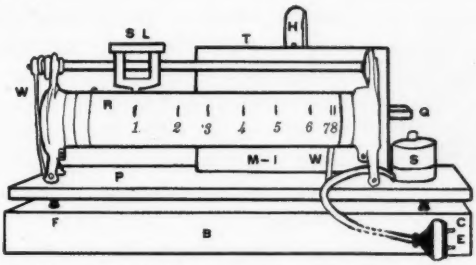


FIG. 2. SCOTT MICRO-INCINERATOR; BACK VIEW

B Base board to which the incinerator is attached. It fits the laboratory lockers.
C E Cap for connecting with the electric current.
F Rubber foot between the insulating plate of the incinerator and the base board.
H Handle of the top insulating material over the quartz tube.
M-1 Insulating material containing the quartz tube.
P Insulating plate to which the incinerator is attached.
Q Quartz plate projecting from the quartz tube. This is to support the slide of sections to be incinerated.
R Rheostat with rider (SL). It is divided into 8 intervals, 1 to 8.
S Switch for turning the current on and off.
SL Rider that may be slid along the rheostat to vary the resistance and thus increase the heating of the coil surrounding the quartz tube.
T Top of the quartz tube insulator with its handle (H).
W W Wire from the electric circuit to the rheostat and the heating coil about the quartz tube.

An American student, Dr. Gordon H. Scott (1933) of Washington University, St. Louis, who has had the advantage of studying with Dr. Policard, has passed on to his colleagues the knowledge gained by that association. He has published many papers showing the advantages of the incineration method and has devised an improved form of micro-incinerator (Figs. 1 & 2) with which any histologist can make

sur une lame de verre mince, et on examine ou on en mesure même les compartimens cellulaires au microscope. On place ensuite avec précaution sur le feu cette petite lame que l'on fait chauffer au rouge pendant quelque temps. On la retire en l'éloignant peu à peu et graduellement de la chaleur. En l'observant alors au microscope, on croirait que ce tissu n'a nullement été altéré et que son organisation est restée intacte."

his own incinerations and see for himself the amount and location of the minerals in the structures he is studying.

Fixation of Specimens for Incineration

In fixing tissues and organs for micro-incineration it is self-evident that the ideal fixing agent should neither add nor remove any of the mineral matter. The fixer almost universally advocated is: absolute ethyl alcohol, 90 parts; neutral strong formalin, 10 parts. Other alcohols alone or with the addition of formalin may be used, for example: propyl alcohol (propanol), butyl alcohol, and dioxan (diethylene oxide). These fixers have the further advantage that warm paraffin is soluble in them, therefore no special clearing agent like xylene or cedar oil is necessary, but the tissue can be transferred directly from the dehydrating fixer to the warm paraffin.

Unfortunately, the above fixers do not give the best histological details, hence experiments were made with some of the best known fixers in microscopic anatomy, for example (1) 3% aqueous solution of dichromate of potassium with the addition of 10% neutral strong formalin, and (2) Helly's fluid.

If small masses of tissue (1 cc.) are fixed in one of these fixatives and then thoroly washed in running water for 8 to 10 hours, then in 67% alcohol with frequent changes until no more color appears in the liquid, there will be left only a mere trace of the chromium; and with the Helly's fluid the mercury can all be removed by the use of iodin in the alcohol, and later by the use of iodized 82% alcohol after the paraffin has been removed by xylene from the dried sections.

To make sure of the presence and amount of the chromium which might remain in the tissues, the whole liver of a rat that had been fixed in the dichromate-formalin mixture was analyzed by Dr. J. B. Sumner, biochemist at Cornell University. He found only the smallest trace of chromium remaining, an amount so small that in a microscopic section it would be practically negligible. One must remember, however, that with dichromate in the fixer there may be a trace remaining in the ashes. The only reason for using fixers other than alcohol and formalin is the definiteness that they preserve in the histological elements and the greater excellence of the stained preparations.

Dehydrating, Clearing, Embedding and Sectioning

For dehydrating, absolute ethyl alcohol should be used; but if propanol, butyl alcohol or dioxan is used for a fixer, the full strength without formalin should be used plentifully to eliminate all the water. If absolute alcohol is used for dehydration, the specimens may be

cleared in xylene or cedar oil. If the other alcohols or dioxan is used, the tissue can be transferred directly to melted paraffin. For the infiltration before embedding, it has been found advantageous to begin with a low melting point paraffin (40–45°) and to finish with one of 56–58° C. In practice, one soon finds that the harder the paraffin and the cooler the room, the thinner can the ribbons of sections be cut, always providing that a good microtome and a sharp knife are available.

The sections must be thin for successful incinerations; 3–10 μ represent the limits for most tissues. If the tissue becomes too hard by alcohol fixation, so that ribbons cannot be cut, the method of W. J. G. Land (1915) of Chicago University (also in Chamberlain, 1932, p. 125) can be employed. This method consists in cutting away the paraffin until the tissue is uncovered at one end, then soaking the block in cold water for a day or more. This treatment made it possible for the author to cut ligamentum nuchae in ribbons 3–7 μ thick while, if not so treated, the tissue was so hard that it was torn out of the paraffin block when trying to cut it.

For spreading the sections, nothing is so good as distilled water on the slide, warming the slide on a heating plate. Needles are a help for straightening the warmed sections. The slides must be sufficiently hard so as not to soften in the heat required for burning the paraffin and organic matter away. I have found Corex D glass slides or those made of Pyrex most satisfactory. For cleaning them, the Bon Ami method of Stitt (1923, 1927) is used; then the water spreads out in an even layer over the slide. After the sections are spread they must be allowed to become perfectly dry either in the air or in a drying oven. If moisture remains under the sections they are likely to become loosened from the slide in the heating.

No albumen is used on the slide, and unless mercury is used in the fixer, the paraffin need not be removed before incineration.

The Process of Incineration

The slide bearing the sections to be incinerated is placed on a quartz plate and moved to the middle of the quartz tube (Figs. 1 & 2, Q). Then the lid (C) is closed over one end, the insulating top put in place (Fig. 2, T) and the electric current turned on.

Each incinerator should be calibrated by the manufacturers, and the intervals plainly marked on the rheostat. In Fig. 2 these are indicated by the numerals 1 to 8.

The heating must be gradual. Ordinarily the first interval is 10 minutes, and the succeeding ones 5 minutes each except the 8th which

is one minute. This brings the heat up to 637° C. (see Table 1) and the quartz tube will be red hot thruout its length.

One can tell only by trial the heat necessary to burn off all the organic matter in a given tissue. Some require more heat than others and, as the necessary heating has not been standardized, each experimenter must determine for himself the best amount of heat. For beginners it is good practice to use all the intervals; then turn off the current, remove the insulating top and open the cover at the end of the quartz heating tube so that the air can circulate freely thru the hot tube. In about half an hour the quartz slip bearing the slide of sections can be removed with forceps. The slide will cool faster in the open air. If the sections on the slide look white or gray, the heating has been sufficient, but if they look brown not all the organic matter has been burned.

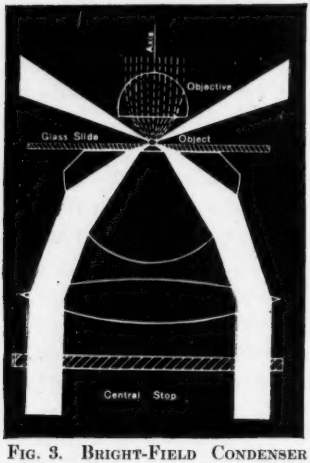


FIG. 3. BRIGHT-FIELD CONDENSER WITH CENTRAL STOP TO GIVE DARK-FIELD ILLUMINATION

This is a sectional view showing the hollow cone of light focusing on the object and then continuing wholly outside the aperture of the objective.

The light deflected by the object into the objective is represented by broken lines.

The glass slide is in homogeneous contact with the top of the condenser and the medium beyond the object is represented as homogeneous with glass. (From *The Microscope*).

COVERING INCINERATED SPECIMENS

The ashes remaining must not be touched, as the mineral matter is easily disturbed and only a chaotic mass would result. To protect the ashes, use a cover somewhat larger than the series on the slide. Hold it in place with the thumb and finger of one hand, then heat a wire or some metal rod in the Bunsen flame until quite hot, then put the wire on a mass of beeswax until it

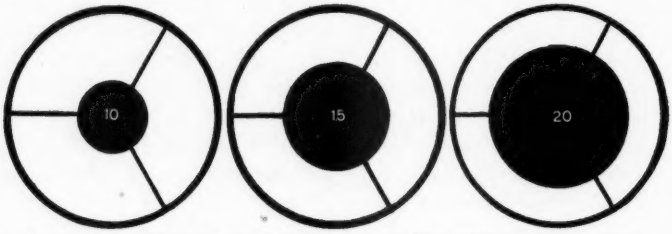
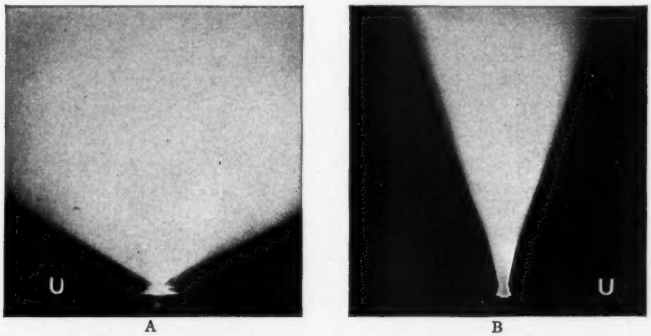


FIG. 4. DARK-STOPS OF 10, 15 AND 20 MM. TO USE WITH THE REFRACTING CONDENSER. (From *The Microscope*.)

is well covered with the melted wax. The wire is then moved along the edges of the cover glass. The wax will harden almost instantly and make a good seal. Later this wax can be covered with shellac cement to make a harder and more permanent seal. This mounts the object in air. If one wishes to see how the ashes would look if mounted in a resin or in petrolatum, a tiny drop of xylene balsam or refined mineral oil (petrolatum) can be added to one of the sections before putting on the cover glass. One can then compare the appearance of the mineral matter mounted in air and in a liquid. It will be seen that those in air show greater detail.

Dark-field Microscope for the Study of Incinerations

A dark-field shows the mineral particles much better than a light-field. The special paraboloid or reflecting dark-field condensers may be used, but these are mostly designed for high powers, the field



A B

Fig. 5. A. Full cone of light from a 1.40 N. A. condenser shown in fluorescent glass (U). This makes clear that for the dark-field hollow cone (Fig. 8), the iris of the substage condenser must be wide open.

B. Cone of light from the same condenser with the substage iris open only 10 mm. If the condenser iris is closed like this, there could be no hollow cone for a dark-field. (Both figures are from *The Microscope* and of natural size.)

lighted is very small, and the thickness of the slide must conform accurately to the focus of the condenser. For the study of micro-incinerations a large field as well as a small one is almost a necessity. It was found by experiment that the ordinary refracting condenser of the Abbé or the aplanatic type answered for all powers by using the appropriate central, dark-stop below it (Fig. 3), and by using objectives above the 16 mm. (10x) with an iris diaphragm to reduce the aperture the correct amount, the reduction necessary being easily determined by trial. The central dark-stops (Fig. 4) permit only a shell of the outer rays of great aperture to pass on to the focus. They are of an aperture so great that if the iris of the objective is closed the

right amount none of the direct light can get into the objective, and the object is then seen by the light reflected or diffracted by it into the microscope (Fig. 3). Consequently the object will appear bright, but the back-ground will be dark.

In all dark-field observation, the slide bearing the specimen should be in immersion contact with the top of the condenser.

The dark-stops necessary for all objectives are shown in Fig. 4. If the central stop is too large, the shell of light (Figs. 5 & 6) will be too thin and the object at the focus will not be sufficiently lighted; if it is too small, some of the inner marginal rays will pass into the objective and render the field wholly or partly light, that is, the object will be seen partly by bright- and partly by dark-field effects. In most cases this is confusing. The same effect may be produced by opening the objective iris too much with the higher powers.

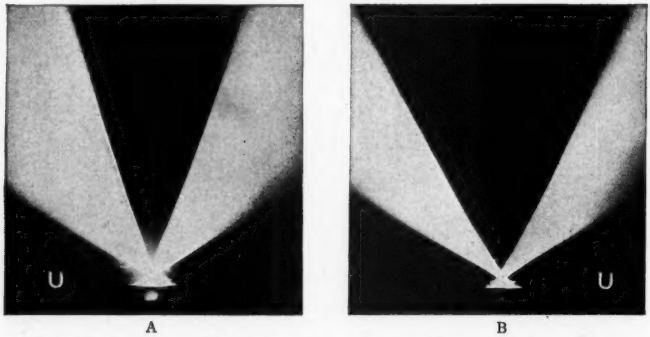


FIG. 6. A. Hollow cone of light shown in fluorescent uranium glass (U) with a substage, dark-stop of 10 mm. A and B natural size.

B. Hollow cone with a dark-stop of 20 mm. diameter. This refracting condenser was rated with an aperture of 1.40. (From *The Microscope*) (See also Fig. 5.)

LIGHT FOR DARK-FIELD MICROSCOPY

With the dark-field microscope, a brilliant source of light is necessary as only that reflected or diffracted by the object finally enters the objective (Fig. 3). In figures 7 and 8 are shown two lamps giving daylight quality illumination by the aid of the Corning Glass Works' 'daylite glass screens.' For the 16 mm. and the 8 mm. objectives, the Chalet Lamp answers well for dark-field, and for bright-field with all powers. But for the 4, 3 and oil immersion objectives a higher illuminant is necessary. The 6 volt, 108 watt research lamp (Fig. 8) answers well. Bright sunlight might be used, of course, but that is not always available.

Method of Study of Micro-incinerations; Comparison with Neighboring Stained Sections

In spreading the series for micro-incineration it has been found of great advantage to put every other slide of the series on ordinary, albumenized slides for staining. Then for the study, the incinerated preparation is put under the dark-field microscope, and the stained section under a bright-field microscope. One can then look from one microscope to the other and make sure that practically the same part is being observed in both fields. As one is more familiar with stained sections, the stained one acts as a control.

Calibration of a Micro-incinerator

Table 1 shows the different settings (1–8) of the rider (R) on the resistance coil (Fig. 2), the time of each setting and the millivolts when the micro-incinerator is in the circuit, and finally the temperature centigrade in the center of the quartz tube (Figs. 1 & 2, Q) at the end of each of the 8 settings. In our laboratory the alternating current during calibration was practically constant at 113 volts.

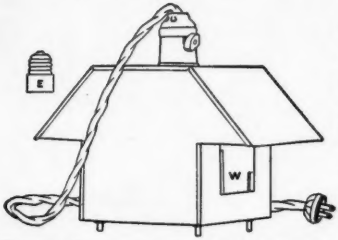


Fig. 7. Chalet Daylight Microscope Lamp.

For bright-field with all powers and for dark-field work with the lower objectives, 16 mm. (10x) and 8 mm. (20x). (From *The Microscope*).

For the determinations, a Chromel-Alumel thermo-couple and a potentiometer with the necessary ammeter and voltmeter were employed.

The observations were made in

Table I. Calibration of a Micro-incinerator

Setting	Minutes	Amperes	Volts	Watts (AxV)	Millivolts	Temperature, ° C.
1	10	1.60	113	180.80	4.32	104
2	5	1.90	113	214.70	6.29	150
3	5	2.18	113	246.34	9.68	235
4	5	2.50	113	282.54	12.80	311
5	5	2.82	113	318.66	16.20	394
6	5	3.25	113	367.25	20.40	492
7	5	3.70	113	418.10	25.00	601
8	1	3.82	113	431.66	26.50	637

the laboratory at Stimson Hall, Cornell University, by Dr. C. W. Gartlein of the Department of Physics. This department also loaned the necessary apparatus.

It will be seen from the table that it takes 25 minutes for the incinerator to increase from room temperature of about 20° to 300° C.; and 15 additional minutes to increase the temperature to 600° C. With each setting there is less resistance in the rheostat, and therefore more current goes thru the heating coil about the quartz tube. It is

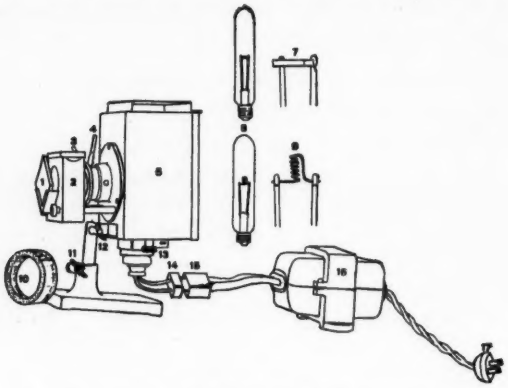


FIG. 8. ADJUSTABLE, 6-VOLT, 108-WATT RESEARCH LAMP

(Modified from the Bausch & Lomb Optical Co.'s catalogue.)

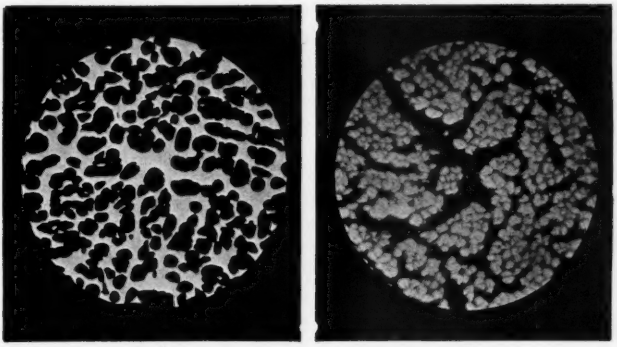
1 Daylight-glass screen.
2 Frame for holding water cell.
3 Handle for opening and closing the iris diaphragm.
4 Handle for focusing the condenser.
5 Lamp-house.
7 Ribbon-filament of the upper cylindrical lamp.
8–9 Coil-filament and cylindrical lamp.
10 Water cell of a glass tube with heat absorbing faces (Dr. H. P. Gage).
11–12 Set screws for the inclination and elevation adjustments.
13 Adjusting screws for centering the lamp.
14–15 Mistakeless connection for the lamp cable.
16–17 Step-down transformer (110 to 6 volts), and connection for the 110-volt circuit. (From *The Microscope*.)

also evident that with an adjustable rheostat one can regulate it at will to heat slowly or rapidly depending upon the position of the rider (SL). In Fig. 2 it is readily seen that the farther to the left the rider (SL) is placed, the more of the resistance in the rheostat (R) is used and hence the less the heating in the coil about the quartz tube, and the slower the heating. Conversely, of course, the farther to the right the rider is placed, the less the resistance and the greater the current for the heating coil. One can also regulate the heat somewhat by taking off the insulating top (Fig. 1, T) and opening the cover at one

end of the quartz tube (C). This lessens the insulation and allows a freer circulation of air thru the quartz tube.

As tissues differ in ease of incineration it behooves one to know all the possibilities and, therefore, to make necessary modifications.

In the ordinary room temperature of 20° C. if the insulating top is removed and cap (C) turned back as in Fig. 2, it takes about 40 minutes for the furnace to cool down from the red heat to 300° C., and then 85 minutes more to reach 34° C.



A B

FIG. 9. A. Section of elastic fibers in the ligamentum nuchae of the ox. The elastic fibers were stained black by Verhoeff's iron hematoxylin method. The intervening collagenous tissue is unstained.

B. Incinerated section showing the mass of mineral matter in each elastic fiber. Both A and B are magnified 250 diameters. (From *The Microscope.*)

As stated above, each micro-incinerator should be fully calibrated by the manufacturer, giving all the data as shown in the accompanying table for the special incinerator used for this article.

REFERENCES

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GAGE, S. H. 1936. The Microscope, 16th ed. In chapter xiv is given the methods of incineration, and the apparatus needed. On pp. 405–9 is given the method of cleaning slides and cover-glasses with Bon Ami, and on p. 478 the Land method of softening hard material by soaking the paraffin-imbedded object in cold water.

LAND, W. J. G. 1915. Microtechnical methods. Contributions from the Hull Botanical Laboratory No. 203. Bot. Gaz., **59**, 397–401. On p. 401 under the heading, Softening Refractory Material Imbedded in Paraffin, Land gives the device of soaking the imbedded material in cold water to render it sectionable. See also Chamberlain.

LIESEGANG, R. E. 1910. Die Veraschung von Mikrotomschnitten. Biochem. Zts., **28**, p. 413 *et seq.* He used sections 20 μ thick on a glass slip and heated the slip over a Bunsen flame. Such sections do not give good results.

POLICARD, A. 1923. Sur une méthode de micro-incinération applicable aux récherches histochimiques. Bull. Soc. Chim. de France, 4th ser., **33**, 1551.

————. 1923. La minéralization des coupes histologiques par calcination et son intérét comme méthode histochimiques générale. Compt. Rend. Acad. Sci., **176**, 1012. In these first papers Policard describes his incinerator and gives explicit directions for the procedure in micro-incineration.

————. 1932. Some new methods in histochemistry. The Harvey Lectures delivered under the auspices of the Harvey Society of New York, 1931–32, under the patronage of the New York Academy of Medicine. 204–26. In this lecture Policard describes the micro-incineration process, but points out that it is only the first step in the analysis of the minerals present. Chemical microscopy must be applied, and the quartz spectrograph, for a complete analysis, the methods for which he gives.

POLICARD, A. and OKKELS, H. 1930. Localizing inorganic substances in microscopic sections. The Micro-Incineration Method. Anat. Record, **44**, 349–61. This paper contains a picture of Dr. Policard's micro-incinerator and some excellent photomicrographs of incinerated specimens with a good discussion of the process and the findings. There is also an extended bibliography.

RASPAIL, FRANCOIS-VINCENT. 1833. Nouveau système de chimie organique fondé sur des méthodes nouvelles d'observation. p. 528, *et seq.* Raspail has been claimed as the founder of chemical microscopy or microchemistry. He applied the microscope to the study of plant and animal structures with great success, and among his méthods was that of incineration to show the presence of mineral matter in soft tissues after the organic matter had been burned away.

SCOTT, GORDON H. 1933. A critical study and review of the method of micro-incineration. Protoplasma, **20**, 133–51. This paper is full of useful information concerning the history and method of incineration, and contains 74 references in all fields where micro-incineration has been applied.

————. 1933. The localization of mineral salts in cells of some mammalian tissues by micro-incineration. Amer. J. Anat., **53**, 243–79. This paper contains 46 figures of micro-incinerations in three plates and gives references to 46 other papers, including those of special historical interest.

SCOTT, GORDON H. and WILLIAMS, PHILIP S. 1935. The spectographic analysis of biological material. Anat. Record, **64**, 107–27. This is a continuation of the method of histospectography insisted on by Policard (1932).

STITT, E. R. 1923, 7th ed.; 1927, 8th ed. Practical Bacteriology, Blood Work and Animal Parasitology. The Bon Ami method of cleaning glass for microscopical purposes appears on p. 299 of the 7th ed. and on p. 314 of the 8th ed.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi — *Abstract Editor*

MICROSCOPE AND OTHER APPARATUS

HANCE, R. T. **"Air conditioning" for microtomes.** *Science*, **86**, 313–4. 1937.

It is well known that hot weather interferes with paraffin sectioning. The author's scheme proposes to better working conditions so as to make cutting much easier and effective. Select a 12x12x18 in. corrugated cardboard box. Lay it on one side with the top opening toward you and top flaps intact. Slide the microtome in it and cut a 2.5x4 in. window in the box just above the object block. Punch a few holes in the bottom and top of a pound size coffee can and insulate the sides with felt or paper. Put 1–2 lbs. dry ice in this tin and place it over the window in the box. A down draught of air flows thru the holes over the ice and downward over object and knife. With lid flaps closed, the inside temperature soon falls 6–8°C. below room temperature. Removal of the bottom side of the box facilitates handling of the microtome. Two lbs. of dry ice cost ten cents and adequately air condition a microtome for 5–6 hr.—*J. A. de Tomasi.*

HOPKINS, D. L. **The relation between temperature and locomotion in the marine amoeba, Flabellula mira Schaeffer, with special reference to adaptation to temperature.** *Protoplasma*, **28**, 161. 1937.

Apparatus is described for controlling the temperature of an object under the microscope with a fluctuation of ± 0.2°, if the desired temperature is between 0° and 50°C. The cooling device consists of a reservoir containing a Lolag hair-pin heater, an Aminco thermostatic thermoregulator with a relay and rectifying transformer. For low temperatures ice, with salt if necessary, is placed in a central chamber in the reservoir. The temperature chamber made to fit the microscope stop is a metal jacket about a central chamber, with glass above and below,and with external levers to move the object container in the central chamber. An electric motor pump drives the water thru tubes connecting the cooling device to the temperature chamber, with a thermometer chamber interposed.—*R. Chambers.*

PHOTOMICROGRAPHY

HADEN, R. L. **A photographic method for visualizing the shape of the red blood cells.** *J. Lab. & Clin. Med.*, **22**, 1262. 1937.

The method used is not new to expert photographers, except that its use in the case of blood seems to have passed heretofore unnoticed. The principle has frequently been resorted to in producing so-called bas-relief in photographs of macroscopic objects, also with glass x-ray plates to give a stereoscopic effect in the composite picture. If the positive and negative plates are carefully matched, with their glass surfaces in contact, the result is an effect of depth in the image. Held together with airplane cement, the plates can be used as slides for projection or for making prints.—*J. A. de Tomasi.*

DYES AND THEIR BIOLOGICAL USES

BENIANS, T. H. C. **Observations on the action of Congo red on normal and leucemic blood.** *J. Lab. & Clin. Med.*, **22**, 1246–51. 1937.

Results are reported of investigations dealing with the action of Congo red on blood cells and body fluids. It is found that a 2% soln. of the dye has no apparent effect on normal blood constituents, but in the case of myelogenous leucemia with

a high white count it causes the precipitation of a thick, elastic gelatinous mass. This gel has no definite structure; it does not show syneresis upon standing, and retains the dye for some time when submersed in water. The blood cells suspended in the gel do not show any definite arrangement. The gel formation is considered to be related to the presence of ruptured leucemic cells, since the serum alone will not cause precipitation. Thus this reaction, altho not necessarily specific, can be used as a simple clinical test for the diagnosis of active leucemia. The reverse of the action of Congo red, an acid dye with the colored anion negatively charged, is obtained when an electropositive dye, methyl violet 6B, is added to the same leucemic blood. The acid dye, Congo red, acts as a dispersing agent of both the cell units and their contents, the basic dye as an aggregating agent. Congo red is also found to prevent coagulation, phagocytosis and hemolysis, but has no action on the sensitization of cells and the agglutination of erythrocytes and bacteria. These effects are again attributed to the dispersing action of the dye on colloidal particles in the cellular system.—*J. A. de Tomasi.*

BLUM, H. F., PLACE, N. and GARRETT, R. L. **Photodynamic hemolysis. I. The effect of dye concentration and temperature.** *J. Cell. and Comp. Physiol.*, **9**, 217–28. 1937.

Rose bengal acts as a simple lysin in the dark; at low concentrations it acts as a lysin only in the presence of light. The mechanisms are very different as is shown by the effect of temperature. In the absence of light, changes in temperature produce irregular effects, but at concentrations at which hemolysis occurs only in the presence of light the Q_{10} is quite regular, having a value of 1.2.—*Authors' Abstract.*

BLUM, H. F. **Photodynamic hemolysis. II. Modes of inhibition.** *J. Cell. and Comp. Physiol.*, **9**, 229–40. 1937.

Photodynamic hemolysis may be inhibited by: 1) removal of O_2 or introduction of reducing substances, and 2) by introducing substances which prevent the combination of the dye with the cells. Failure to recognize this has led to erroneous conclusions. The alleged 'optical desensitization' by phenosafranine is probably an inhibition of the second type.—*Author's Abstract.*

BORRISS, H. **Beitrage zur Kenntnis der Wirkung von Elektrolyten auf die Färbung planzlicher Zellmembranen mit Thioninfarbstoffen.** *Protoplasma*, **28**, 23. 1937.

The addition of KNO_3 (in tap water pH 7.4) to *Allium* epidermal strips with cell walls previously stained with methylene blue gives rise to color changes and colloidal phenomena which may be ascribed to colloidal modifications of methylene blue nitrate. Chlorides of the alkali and alkaline earth metals do not produce the colloidal effects. Thionin-hydrochloride, di-, tri-, and tetramethylthionin did not produce similar coacervates.—*R. Chambers.*

CHAMBERS, R. and KEMPTON, R. T. **The elimination of neutral red by the frog's kidney.** *J. Cell. and Comp. Physiol.*, **10**, 199–222. 1937.

The elimination of neutral red in perfusion experiments with the frog's kidney is increased by increasing the acidity of the urine. Suppression of its elimination is obtained through alkalinization of the urine by perfusing either 1) the aorta or renal portal vein with 1.0% $NaHCO_3$ in Ringer (pH>8.0) or 2) the renal portal vein with 0.6% NH_4Cl in Ringer (ca. pH 7.2). Enhancement of its elimination is obtained through acidification of the urine by perfusing the aorta with NH_4Cl in Ringer (ca. pH 7.2). The inhibiting effect of KCN or of ethyl urethane is due to their alkalinization of the urine and can be nullified by superposing an aortic perfusion of NH_4Cl-Ringer which acidifies the urine. Neutral red, stored in the cells of the tubules, can be caused to be eliminated with an aortic perfusion of NH_4Cl-Ringer. The elimination of neutral red may be explained in accordance with the known property of the dye of diffusing thru a membrane from an alkaline to a more acid medium. Its passage thru the walls of the tubules does not depend upon the metabolic activity of the tubule cells. These two features place neutral red in a different class from that of phenol red whose elimination is due more nearly to an active, secretory process.—*Authors' Abstract.*

CONN, H. J. **Standardization of tablets for determining methylene blue reduction in milk.** *Amer. J. Pub. Health*, **27**, 793–5. 1937.

American manufacturers are now producing standardized methylene blue tablets, certified by the Biological Stain Commission, to take the place of the variable Danish product. It is recommended that the tablets be made from methylene blue thiocyanate instead of chloride, and contain 7.5 mg. each, giving a dilution of 1:300,000 in milk.—*M. W. Jennison.*

GORDON, J. and WOOD, N. **Reversible neutralization by Congo red of the anthracidal power of serum.** *J. Hygiene*, **37**, 471–3. 1937.

The senior author had previously shown that Congo red will inactivate complement and several antibodies, and that the effect could be reversed by adding charcoal or some other agent on which the dye was adsorbed. Antiserum bactericidal for *B. anthracis* was readily inactivated by Congo red and the reversal, or liberation of anthracidal bactericidins was ingeniously accomplished by treatment with serum from which the anthracidal power had been removed with charcoal. It is considered probable that since Congo red at the pH of serum is loosely bound with protein this reaction indicates the presence of chemical groups like those of proteins in complement and in bactericidins.—*M. S. Marshall.*

GUTSTEIN, M. **Zur Theorie der Vitalfäbung. Ztsch. exper. Med., 82,** 479–524. 1932.

The results of lengthy investigations on vital dyes and stains and a correlation of many data derived from various sources can be summarized as follows: In yeasts, as well as in thymus cells, vital staining appears to be effective in decreasing degrees, according to the series: Victoria blue > fuchsin > safranin > methyl violet > Nile blue. Vital staining of yeasts by basic dyes is a function of several factors: their relative solubility in lipoids, their sensitivity to reduction by plant cells, their susceptibility to precipitation by water-soluble phosphatids. Among the acid dyes, on the other hand, those soluble in lipoids are most easily taken up by the yeast cell. Colloidal dyes like Congo red, trypan blue, and brilliant purpurin R are also taken up in relatively appreciable amounts. Also in the case of acid dyes, reduction by plant cells is a limiting factor in vital staining; and water-soluble phosphatids will not precipitate them. Acid dyes, which are not soluble in phosphatids, enter the cells, (e. g., paramecia) as free acids (phosphatid-soluble). The granules appearing in vitally stained animal cells are not artefacts but true cell structures. The different behavior of acid and basic dyes is interpreted as dependent upon their respective mode of joining the cell constituents: the former are taken up mechanically as solutions, the latter enter into a closer combination of chemical or electropolar nature. The distribution of acid vital dyes in animal cells is governed by a definite fall of the pH, which suggests a difference in reaction between the inside and outside surface of the cell membrane.—*J. A. de Tomasi.*

MADISON, R. R. and SNOW, J. E. **Effects of surgical antiseptics on streptofibrinolysin.** *Proc. Soc. Exp. Biol. & Med.*, **36**, 592–5. 1937.

Twenty-eight antiseptics (including acriflavine, gentian violet, picric acid, tannic acid) were found to inhibit the clotting of solutions of fibrinogen by thrombin. Acriflavine and gentian violet prevented the normal spontaneous autolysis of human fibrin, as did 4 of the remaining 26 substances tested. Gentian violet and tannic acid in the dilutions tested reduced the activity of streptofibrinolysin on human fibrin.—*M. S. Marshall.*

THORNTON, H. R. **Modified methylene blue reduction technique.** *Amer. J. Pub. Health*, **27**, 791–2. 1937.

It has been proposed that in the methylene blue reduction test for determining the sanitary quality of milk, the tubes be shaken during incubation. The author shows that shaking does not appreciably increase the accuracy of the test. The proposed modification might, on the other hand, complicate the test to the extent that uniform shaking technic would be improbable.—*M. W. Jennison.*

ANIMAL MICROTECHNIC

BARBROW, A. L. An improved stain for use in rapid biopsy diagnosis. *J. Lab. & Clin. Med.*, **22**, 1175. 1937.

For speed and accuracy the following stain is considered eminently satisfactory. Being a combination of Van Gieson's stain and Weigert's Fe hematoxylin stain, it consists of 3 solutions (A, B and C) mixed, respectively, in 5:2:10 proportions. *Sol. A:* thoroly ripened 1% hematoxylin in 95% alcohol. *Sol. B:* liquor ferri sesquichloratis, 4 cc.; HCl (no conc. given), 1 cc.; water, 95 cc. *Sol. C.* 1% aq. acid fuchsin, 10 cc.; sat. soln. picric acid, 100 cc. After mixing, this combined stain is ready for immediate use but does not keep. Flood the frozen section 1 min., rinse, examine wet or mounted in balsam.—*J. A. de Tomasi.*

BUSNEL, R. G. Note relative aux techniques employées pour la recherche des cellules à mucus. *Bull. d'Histol. Appl.*, **14**, 205–7. 1937.

Generally, methods used for the study of mucus cells are based on their affinity for carmine. The author in his studies on fish gills and skin has experimented with metachromatic dyes: thionin, toluidine blue, methylene blue, methylene violet, methylene azure. One sample technic follows: Fix in picro-formol, embed, cut and run down to water. Wash 10 min. in dist. water, dip 1–2 min. in 0.5% aq. toluidine blue. Pass rapidly thru abs. alcohol, clear and mount. Mucus cells appear violet against a dark blue background. Cartilaginous tissue may appear mauve color and the erythrocytes green.—*J. A. de Tomasi.*

CRETIN, A. Note sur les colorations par des colorants-mordants spécialement par l'alizarine-hématoxyline phosphotungstique après mordancage triple. *Bull. d'Histol. Appl.*, **14**, 163–7. 1937.

This technic devised for staining bone material indicates a new departure from usual methods. It is based upon the principle of depositing lakes from 2 contrasting dyestuffs, following a triple mordanting. The fixative is made up as follows: Add enough 40% formol to 1 liter of commercial fuel alcohol to obtain a concentration of 50% alcohol; saturate with picric acid; add 3 g. NaCl and 250 cc. of 15% trichloracetic acid in same grade of alcohol. Fix 4–5 days. Decalcify, if necessary, in 15% aq. NaCl containing 5% HCl. Embed in paraffin or celloidin. Make up the following mordant solution: 15% $AlCl_3$, 150 cc.; 3% $FeCl_3$, 25 cc.; dist. water, 200 cc.; mix; add 30% $CaCl_2$, 450 cc., and make up to 1 liter with dist. water. Dilute 1:2 with dist. water, and apply 36 hr. or more. Wash thoroly. Stain 1–24 hr. according to intensity desired in the following (remembering the staining procedure is progressive): 3% hematoxylin, iodate-ripened, 100 cc.; 10% alizarin red S, 150 cc. At time of staining add 10% phosphotungstic acid, 1 cc., to 25 cc. of the stain. Use after 24 hr. and for no longer than 8–10 days. Wash thoroly in dist. water, several times in 95% alcohol, in dioxan, and in tap water. Dehydrate and mount.

Various color combinations are obtained from the 3 metal ions in this mordant: With hematoxylin the Ca-Al lake yields light blue to Prussian blue, the Fe-Al lake, blue violet to violet and the Fe-Ca lake, violet black; with alizarin the colors shown by these 3 lakes are, respectively orange red to bright red, dark orange, and reddish violet.—*J. A. de Tomasi.*

CROSSMON, G. A modification of Mallory's connective tissue stain with a discussion of the principles involved. *Anat. Rec.*, **69**, 33–8. 1937.

A microscopically controlled Mallory method for producing constant results is given. The original method called for a general staining of the sections with acid fuchsin. Phosphomolybdic acid then acted to remove the stain from the collagenous fibers and acted as a mordant for the subsequent staining of these fibers with anilin blue. Orange G was used with the anilin blue to bring out red blood corpuscles and myelin sheaths. Mallory combined the phosphomolybdic acid with anilin blue and orange G. This prevented microscopically controlling the decolorization of the collagen fibers stained with acid fuchsin. This fault is overcome in the following method:

Fix in Zenker's fluid. Cut in paraffin. Remove bichloride in modified Lugol's solution. (Iodine, 2 g.; potassium iodide, 4 g.; dist. water, 100 cc.) Use 5 minutes

or longer. Several changes of 70% ethyl alcohol to remove iodine. Wash in running tap water 10 min. Stain in Mayer's acid hemalum or in Weigert's iron hematoxylin until nuclei are overstained. (This step may be omitted if so desired.) Wash in running tap water 10 min. Stain in the following 1 min. or more: Acid fuchsin (Nat. Aniline Co.), 1.0 g.; orange G (Nat. Aniline Co.), 0.4 g.; dist. water, 300 cc.; thymol crystals, 0.2 g.; glacial acetic acid, 3.0 cc. (This solution keeps for months and is more economical on orange G than the original method.) Rinse in dist. water. Decolorize in 1% aq. phosphotungstic acid solution (or phosphomolybdic acid 1% aqueous) watching progress under microscope until collagenous tissue (such as adventitia of blood vessel) is colorless. Bone and cartilage decolorize less quickly than collagen. Use fresh solution for every second rack of slides since decolorizing ability of acid solution is quickly lost. Rinse in dist. water quickly, thereby keeping the mordanting effect of last step. Stain 5 min. or longer in either of the following solutions: Light green (Krall Microcolor; Eimer and Amend), 1 g.; dist. water, 100 cc.; glacial acetic acid, 1 cc., or anilin blue (Nat. Aniline Co.), 2 g.; dist. water, 100 cc.; glacial acetic acid, 2 cc. Rinse in dist. water quickly, thereby avoiding loss of the connective tissue stain. Differentiate 1–5 min. in 1% acetic acid using microscopic control. Rinse quickly in dist. water and pass directly to the following: Abs. ethyl alcohol, 3 changes; xylene, 3 changes. Mount in balsam. No loss of dye occurs in the abs. alcohol and thus dehydration need not be hurried.—*S. I. Kornhauser.*

GUTSTEIN, M. **New direct staining methods for elementary bodies.** *J. Path. & Bact.*, **45**, 313. 1937.

Elementary bodies can be stained directly by basic dyes without a mordant provided alkaline solutions are used. The method was used mostly for vaccinia and larger virus bodies. Two alternative technics are offered:

Methyl violet: Spread a film of suspension on a carefully cleaned slide. Dry films in air or incubator. If much protein is present, rinse film with saline, then in dist. water. Fix in CH_3OH 30 min. or more. Place slide in a dry Petri dish. Stain in a mixture of equal parts 1% methyl violet in dist. water and 2% $NaHCO_3$. Filter the mixture at once onto the slide, cover the dish, and incubate at 37° C. 20–30 min. Rinse in dist. water, dehydrate, mount in cedarwood oil or liquid paraffin. Elementary bodies stain light violet.

Instead of the above, basic fuchsin may be used (fuchsin, 1.0 g.; alcohol, 10 cc.; dist. water, 90 cc.). The elementary bodies are pinkish.

Victoria blue: Place inverted slide in Petri dish supported on capillary tubing. Mix Victoria blue 4R, 1.0 g.; alcohol, 10 cc.; dist. water, 90 cc. with an equal amount of 0.02% KOH. Run the filtrate under slide; allow to stand over night. Rinse with dist. water, dehydrate, mount in neutral balsam or paraffin. Elementary bodies stain dark blue.—*S. H. Hutner.*

ITO, T. **Eine einfache Färbungsmethode der Gliazellen mit Eisenchloridalizarinlack nach K. Okajima.** *Folia Anat. Jap.*, 15, 357–9. 1937.

This paper expresses further approval of the "Omnichrom" stain ($FeCl_3$, alizarin lake) developed in 1935 by K. Okajima. The author finds that cells of the nervous system, especially glia cells with their ramifications, stand out distinctly. The schedule is: Fix material 4 days, changing daily, in Regaud acetic (Regaud's sol., 100 cc.; glacial acetic acid, 10–15 drops), or 1 day in Zenker-formol. Mordant 7 days, changing every other day, in 3% $K_2Cr_2O_7$. Wash 24 hr. in running water. Run up and embed in paraffin or celloidin. Stain 5–10 min. in the alizarin lake diluted 1:1. Wash in running water until the section appears reddish violet. Differentiate in 70% or 90% alcohol until no more stain is extracted. Dehydrate in 95% and abs. alcohol, clear and mount. Glia fibers, cytoplasm and chromatin of glia cells and axis cylinders stain violet; nuclear bodies of glia nuclei and erythrocytes, orange red; medullary and myelin sheaths, light yellow.—*J. A. de Tomasi.*

KINGSLEY, D. M. **Dyes for Romanowsky stains.** *J. Lab. & Clin. Med.*, **22**, 1264–73. 1937.

This is a comprehensive review of the literature and work done on dyes and stains of the Romanowsky type since the days of their inception. Emphasis is

laid on some of the less generally known phases of the development of these stains, also on recent progress in this special line of hematology. A long list of references is appended to the article.—*J. A. de Tomasi.*

KRÜGER, O. **Versuche zur Fixierung des Funktionszustandes der Hauptstücke der menschilchen Niere und zur Bedeutung der Kuppenbläschen der Epithelien.** *Zts. mikr.-anat. Forsch.*, **41**, 453–68. 1937.

Four types of preparation of kidneys to preserve the functional state of the epithelium of the convoluted tubules are compared. Measurements were made of the width of the tubules and their lumina and averages given for each method. The size of apical vesicles of secretion formed in the lumina is also discussed and the effect of hypo- and hypertonic salt solution on these vesicles is described. Human kidneys were studied; one subject immediately after execution and two some hours after death due to illness.

Methods used: (1) Slices were cut with a razor and fixed 30 min. in Carnoy's fluid. (2) Perfusion of kidney with body-warm 0.9% NaCl thru renal artery for 20 min., followed by immersion in Carnoy's fluid. (3) Perfusion as above, fixation thru renal artery with Carnoy's fluid. (4) Without salt perfusion but fixation thru renal artery with Carnoy's fluid.

Results: Method 1 gave the tallest epithelium and smallest lumina which were filled with apical vesicles. Method 2 gave wide lumina, low epithelium, few vesicles. Method 3 gave poorer picture than 2 and complete absence of vesicles. Method 4 gave good preparations, but lumina larger than in Method 1. The author believes that secretory vesicles are normally produced at the cell apices. These are preserved and fill the lumina of the convoluted tubules in Methods 1 and 4. If a kidney is perfused with 0.6% NaCl first and then fixed, the vesicles are enlarged and often burst. If, on the contrary, perfusion with 2.5% NaCl precedes fixation, the vesicles are shrunken and angular. The apical vesicles are best preserved by injection of the fixing fluid thru the renal artery without previous salt solution perfusion.—*S. I. Kornhauser.*

LIBER, A. F. **A chromium-hematoxylin stain for myelin in paraffin sections.** *Arch. Path.*, **24**, 230–2. 1937.

This method is presented as an improvement over other methods of staining for myelin in dehydrated material: Fix in formaldehyde, U. S. P.; carry paraffin sections to water; place sections in Weigert's primary mordant (potassium bichromate, 5 g.; fluorchrome, 2 g.; dist. water, 100 cc.) 12 hr.; rinse; stain with Kultschizki's hematoxylin (hematoxylin, 1.5 g.; abs. alcohol, 15 cc.; glacial acetic acid, 2 cc.; dist. water, 100 cc.) 1 hr.; rinse; place sections in 1% lithium carbonate few seconds until blue; wash in running tap water for 1 hr.; place in 0.3% potassium permaganate a few seconds; rinse; differentiate in Pal's solution (1% oxalic acid and 1% sodium sulfite, 1:1); wash in running tap water 1 hr.; dehydrate; clear and mount in balsam.—*L. G. Montgomery.*

RICHTER, D. **Vital staining of bones with madder.** *Biochem. J.*, **31**, 591–5. 1937.

It has been known that bones of animals are colored red by feeding with the root of the madder plant *Rubia tinctorum*, which contains a number of coloring substances related to alizarin. Further investigation shows these substances to be alizarin, ruberythric acid, purpurin, and purpurin-3-carboxylic acid and its glycoside galiosin. Of all these, when fed to young rats and pigeons, only the purpurin carboxylic acid (also its glycoside which is so unstable that it is hydrolized in stomach and intestine) gives a staining of the bright carmin red color typical of madder-stained bones. Bones of rats fed on relatively large amounts of alizarin show a very slight coloring, the color being a pale bluish shade of pink. While it is assumed that in most cases the staining properties depend largely on the physical properties of the tissues on which the stain is adsorbed or in which it is dissolved, it seems from experiments run with bone tissue *in vitro* that in the case of stains related to alizarin, the staining depends rather on their specific chemical affinities for Ca.—*J. A. de Tomasi.*

ROBB-SMITH, A. H. T. **A device to facilitate the impregnation of reticulin fibrils in paraffin sections.** *J. Path. & Bact.*, **45**, 312. 1937.

Paraffin need not be removed from sections before Ag impregnation. The advantages of the following technic include a complete absence of precipitate and a solid impregnation of the finest reticulin fibrils; the results are claimed to be excelled only by the del Rio Hortega "connectivo" technic which is limited to formol fixed frozen sections.

Cut paraffin sections in the usual way and float on warm water which has previously been boiled. Sections are conveniently manipulated on microscope slides with a camel hair brush. To impregnate: 10% NH_4OH, 15 min.; 3 changes dist. water; 0.25% $KMnO_4$, 5 min.; wash in dist. water a few minutes; 1.5% oxalic acid until brown color disappears; 4 changes dist. water; 5% $AgNO_3$, 1 hr. at room temperature; 2 changes dist. water; ammoniacal Ag, 15 min. at room temperature; 3 changes dist. water; 30% formalin, 3 min.; wash in tap water; tone if desired in 0.2% $AuCl_3$, 3 min.; fix in 5% Na thiosulfate, 1 min.; wash and mount as usual, paraffin coming off at xylene stage. To prepare ammoniacal Ag: add 6 drops 10% NaOH to 8 cc. of 10% $AgNO_3$. Add strong NH_4OH until precipitate is just dissolved. Make up to 28 cc. with dist. water. The solution should not smell of ammonia.—*S. H. Hutner.*

SIMMONS, S. W. **Some histopathological changes caused by hypoderma larvae in the oesophagus of cattle.** *J. Parasitology*, **29**, 336–41. 1937.

Place infected oesophageal tissue of freshly slaughtered cattle with intact larvae into Zenker's solution. Prepare sections using paraffin method. Several staining methods may be used to obtain desired effects. Eosin-methylene-blue proves very effective for demonstrating infiltrating leucocytes. For best results stain in a 4% aq. solution of eosin Y for 20 min. Counterstain with Goldhorn's polychrome methylene blue for 15 min.; differentiate in 95% alcohol containing a few drops of rosin solution I. Counterstain hematoxylin with picro-fuchsin (VanGieson) to differentiate connective tissue. Ordinary Delafield's hematoxylin-eosin may also be used.

To estimate the number of eosinophils per cubic millimeter of tissue, count all the cells falling in an area of a crossline ocular micrometer. Compute the square area of ocular micrometer for each power of the microscope. The thickness of the tissue must be known. Compute densities per cubic millimeter of tissue by using the formula: $\frac{D}{Ta} = \frac{N}{1}$ or $N = \frac{D}{Ta}$, in which: a = area in which cells are counted, T = thickness of section, D = cell population of area counted, N = calculated number of cells per cubic millimeter of tissue.

Larvae occur in submucosa and cause inflammatory edema. Edema distorts submucous connective tissue and occasionally invades the muscularis mucosa. Infiltration of leucocytes into the pathological area is striking. Most of the hemocytes are eosinophils, lymphocytes, and plasmocytes. In some cases eosinophils are also myelocytes.—*E. Bachelis.*

TANG, F. F. and WEI, H. **Morphological studies on vaccinia virus cultivated in the developing egg.** *J. Path. & Bact.*, **45**, 317–23. 1937.

Supravital staining is helpful in studying virus-induced lesions in the chorioallantoic membrane of the chick. A piece of infected membrane is laid on a slide, ectodermal surface uppermost, washed with Tyrode's solution, blotted, and a drop or two of 1% cresyl blue or neutral red added to cover it. The slide is then placed in the incubator 5 min., a cover glass applied, and excess fluid removed. By this technic, smaller inclusion (Paschen ?) bodies are faintly blue or violet, while larger (Guarnieri ?) bodies stain irregularly. These structures were seen by darkfield; in methanol fixed Giemsa stained smears; and in untreated material.—*S. H. Hutner.*

WEIBER, I. **Staining method for nasal smears for eosinophile counts.** *J. Lab. & Clin. Med.*, **22**, 1286. 1937.

With this method it is easy to distinguish eosinophiles from neutrophiles, as the former cells stand out bright pink on a blue background while the latter stain

blue. Stain 1 min. with undiluted Wright's stain. Dilute with dist. water until metallic sheen appears. Allow to stand 4 min. Wash with dist. water. Decolorize 2–3 min. with 95% alcohol. Wash with dist. water. Counterstain 2 min. with Loeffler's methylene blue. Wash with dist. water and dry.—*J. A. de Tomasi.*

WILLIAMS, W. W. **Examination of suspected semen stains for spermatozoa.** *J. Lab. & Clin. Med.*, **22**, 1173–5. 1937.

The author claims the following method gives positive results in identifying and differentiating spermatozoa of different species as well as different individuals within the same species. Cut a small piece of the soiled cloth, not more than 1 in. in diameter. Transfer to a clean slide, wet with a few drops of saline and scrape off the surface of cloth with the blunt edge of a scalpel. Remove the cloth and spread the fluid over the slide. Dry, fix with heat and stain by covering film 5 min. with acid 1% Wollschwarz sol. (G. Grübler & Co.) made as follows: 1% aq. Wollschwarz sol., 4 cc.; 2% H_2SO_4, 0.05 cc. Wash. Counterstain 6–8 sec. with Loeffler's methylene blue diluted 1:15. Wash with dist. water. Dry and examine under oil immersion. Heads of spermatozoa stand out as bright golden or yellowish spots against the dull grayish color of the rest of the sperm cell.—*J. A. de Tomasi.*

WRIGHT, R. D. **The blood supply of newly developed epithelial tissue in the liver.** *J. Path. & Bact.*, **45**, 405–14. 1937.

An injection fluid usable at lower pressures than feasible with gelatin masses is prepared by staining olive oil red with Sudan III or blue with a commercial oil-soluble pigment. To these oils add 1/10 weight of oleic acid and 1/40 weight of triethanolamine. Mix 10 volumes of oil with 100 volumes of milk.

The organ studied (liver) was then fixed with formalin via the bile duct for 1 day. Frozen and paraffin sections were made. Frozen sections were unstained. Gelatin injected specimens were cleared in xylene and mounted in balsam; oil injected specimens were cleared in Farrant's medium.—*S. H. Hutner.*

ZHOOKHIN, V. A. **A modification of the silver impregnation method for the staining of reticular latticed fibers.** *J. Lab. & Clin. Med.*, **22**, 1284–6. 1937.

This method for the impregnation of argentosensitive reticulum is said to be rapid, simple and effective: Fix tissue in 1–10% formalin for 6 hr. or longer (or preserve in same for 1 yr. or longer). Wash 1–2 min. in running water; if fixation was prolonged, wash for 1–2 hr. Frozen sections (15–20 μ) are transferred from dist. water to 0.001–0.002% $KMnO_4$ for 2–30 sec. Impregnate 2–3 min. in 20% ammoniacal Ag sol., prepared by adding NH_4OH drop by drop to $AgNO_3$ until precipitate dissolves and then 2–3 drops in excess. Rinse in dist. water and pass to fresh 1–1.2% formalin at pH 7.0. Keep sections in motion with a glass rod and control degree of impregnation under the microscope. Wash, dehydrate and mount. If desired, after the Ag impregnation, the sections may go thru a weakly acid AgCl sol. followed by fixation in 5% $Na_2S_2O_3$ then thoroly washed. Contrast stains (eosin or picric acid) may also be used.—*J. A. de Tomasi.*

PLANT MICROTECHNIC

ECKERT, F. and DOHRER, H. **Die Fortpflanzungsorgane der deutschen Tange.** *Mikrokosmos*, **30**, 92–8. 1937.

The technic suggested in this paper applies to the various seaweeds found on the German coast of the North and Baltic seas. Immediately after collection, immerse the algae in strong Flemming fixative and keep changing until the yellow color of the solution remains unaffected which may require several weeks. Wash in tap water until no more yellow material seems to be extracted. Preserve, if necessary, in: glycerin, 10%; 96% alcohol, 20%; tap water, 70% (keep tightly stoppered). Except with *Himanthalia lorea*, which can be embedded in paraffin and cut 50–60 μ thick, all other algae are handled best in "Cellosol" (Hollborn), as follows: cut material in pieces 1 cm. long, without breaking or mashing the sexual bodies. Flood with 30% alcohol and run up to 96% alcohol at 1 day intervals. Pass thru 3 changes of dioxan, or "Cellosol solvent" (Hollborn). Soak under vacuum 2 days in 1:3 Cellosol and its solvent; 2–5 days in 1:1 of same materials; 8 days in straight Cellosol. Embed in tinfoil boats made by molding the foil around a small box built of cover glasses. After 2–4 days, peel off the foil and

mount the block so that it is fully enclosed in paraffin. Cut 30–70 μ thick. These sections can be preserved indefinitely in 70% alcohol. Four dyes, made by Bayer and marketed by Hollborn, are made into the following filtered staining solutions: erythrosin, sat. sol. in 90% "Hartosol" (Hollborn), or alcohol; iodo-eosin, sat. soln. in 10 cc. dist. water, adding 40 cc. "Hartosol;" light green, same as with iodo-eosin; anilin blue, sat. soln. in 80% alcohol, adding a few drops of glacial acetic acid. Proceed as follows:

A. Paraffin sections: Affix on a cover glass; wash out the paraffin in 3 changes of xylene; dehydrate in 2 changes of methyl alcohol; cover with collodion; stain 10 min. in iodo-eosin; rinse in 90% alcohol; stain briefly in light green until section shows only a slight pink color; rinse quickly in 96% alcohol; dehydrate in 2 Hartosol changes; clear, and mount in "Caedax."

B. Celloidin sections: Transfer to a small watch glass and remove alcohol; cover with dioxan or Cellosol solvent and change several times; pass into denatured alcohol; drain; stain 1 hr. (under cover) with erythrosin; wash off extractable dye with alcohol and stain 20–40 sec. with anilin blue diluted 1:3 with alcohol. Section must appear a blue-red color. Dehydrate quickly with 96% alcohol and several changes in Hartosol; clear and mount as under *A*.

With erythrosin-anilin-blue, or iodo-eosin-anilin-blue, oogonia and antheridia stain a light to deep brown-red; their membranes, light blue; chromatophores and nuclei, dark red; cell wall, slime, and hyphae, blue. Erythrosin-light-green, or iodo-eosin-light-green, stain cell wall, slime, and hyphae green; the other elements as above.—*J. A. de Tomasi.*

GARRETT, S. D. **Brom thymol blue in aqueous sodium hydroxide as a clearing and staining agent for fungus-infected roots.** *Ann. Bot.*, **1**, 563. 1937.

A method for clearing and staining whole roots for direct microscopical examination which is of value in inspecting roots of young plants and detecting infection of the roots of experimental plants is described. The roots are allowed to soak in a solution of 0.04% brom thymol blue in 4% (or "normal") aq. sodium hydroxide for not less than 5 min. Mount in the same reagent. The sodium hydroxide has a macerating action, as well as a clearing effect, which enables the root to be pressed out flat under the cover slip, thus facilitating microscopical examination. Young fungus hyphae, spores and sporangia are stained blue and are easily seen thru the cleared root cortex. The meristematic tissue of the root apices also takes up the blue stain. Material preserved in 70% alcohol appears to respond as well to this treatment as the fresh tissue, but these preparations have no permanent value owing to the continued action of the reagent on the tissues.—*Elizabeth F. Genung.*

MILLER, E. DeW. **A study of the bacterial and alleged mitochondrial content of the cells of the clover nodule.** *Biol. Bull.*, **73**, 112–25. 1937.

Various technics are used by the author to make a clear distinction between the different pleomorphic forms of bacteria and the mitochondria (granular inclusions) in the white and crimson clover nodules. The mitochondrial fixatives used were Altmann's, Regaud's, Champy-Kull's, Flemming's, and Murray's as well as Schaudinn's fluid and iron hematoxylin. All nodules were washed in sterile water before cutting and some were treated before fixation with 95% alcohol or ether for 12–24 hr. Granules and little rods which had the physio-chemical properties of mitochondria became visible after long treatment with 95% alcohol, ether, and fixatives containing 5% of acetic acid.

The morphology of these minute cytoplasmic inclusions in the nodules fails to give enough evidence from their reaction to Janus green, fat solvents, and staining reactions with aniline fuchsin, toluidine blue, and methyl green, to interpret them as mitochondria. Slight variations in technic often change the staining reactions. The results suggest that minute bacterial forms have been confused with mitochondria in the clover nodule and that size, staining, reaction to Janus green and lipoid solvents is not sufficient to differentiate between them.—*W. R. Hunt.*

SEMMENS, C. S. **A technique for mounting filamentous algae.** *Microscope*, (London) **1**, 5–6. 1937.

This procedure, based on the use of glycerin as a mounting medium, is advocated as a useful substitute for more elaborate technics such as the paraffin-Fe

alum hematoxylin, or the Venetian turpentine methods. Results with *Chlamidomonas, Volvox, Euglena, Cyanophyceae,* proved it quite satisfactory. Prepare the following solutions: *Solution 1:* Glycerin, 78 cc.; lactic acid, 80 cc.; phenol, 50 g.; water, 100 cc. *Solution 2:* Soln. 1, 50 cc.; 1% osmic acid, 2 cc.; light green, 0.25 g. *Solution 3:* Soln. 1, 50 cc.; 1% osmic acid, 2 cc.; anilin blue, 0.25 g. *Solution 4:* Soln. 1, 50 cc.; 1% osmic acid, 2 cc.; phloxine, 0.25 g. Partially dry the specimen on blotting paper then drop it for 1 hr. into one of the staining solutions. Remove excess stain with blotting paper and transfer to Soln. 1 for an indefinite time. If a counterstain is wanted, immerse 5–30 min. in any of the other dye solutions, and go back again to Soln. 1. Place a clump of material on a glass plate, tease out one end, cut it off and place on a clean slide without smearing the glass surface. Add a small drop of Soln. 1 and put on a cover slip. Finish the mount with 2 coats of each of 2 ringing varnishes made by dissolving 5 g. gum dammar in 50 cc. chloroform, and in 50 cc. xylene. Before use filter and evaporate to 20 cc.—*J. A. de Tomasi.*

MICROÖRGANISMS

BEERS, C. D. **A method for the sectioning of protozoa en masse.** *Science,* **86,** 381–2. 1937.

This method, for reducing loss of specimens, is based on the use of a bag made from mammalian mesentery for embedding the organisms. Excise a transparent piece of fresh cat or rabbit mesentery 1.5 cm. in diameter. Rinse in warm saline and dip it out with a 2 mm. paraffin bead at the fine end of a wooden pin, so that it hangs symmetrically around it. Stick the pin (head up) into a small cork and tie the mesentery securely with No. 80 black cotton thread. Shake about and float (head down) in Bouin's for 24 hr. so the free edge of the bag stands out and hardens in that position. Wash in 80% alcohol, dehydrate, and dissolve out the paraffin bead for 4 hr. in xylene. Thru abs. alcohol, bring back to 80% alcohol for 24 hr., push the bag with the thread off the pin, and store in same alcohol. Pass the fixed protozoa to 80% alcohol. Make a small basket of fine mesh wire gauze, place in it the mesentery bag and set in 95% alcohol. With a fine tipped pipet, under the dissecting microscope, transfer the organisms to it. The higher sp. gr. of 80% alcohol makes it flow easily into the bag. Close tight and cut off the loose ends. Dehydrate, embed, and section with care not to cut thru the thread.—*J. A. de Tomasi.*

BRYAN, C. S. and NELSON, E. A. **Influence of contaminating bacteria on the results of the microscopic test for streptococcic mastitis.** *Amer. J. Pub. Health,* **27,** 914–7. 1937.

Contaminating bacteria, which may gain entrance to milk samples as a result of faulty technic in collecting the samples, decrease the accuracy of the results of the microscopic test for detecting streptococcic mastitis. Placing 0.1 cc. of a sterile 1:500 aqueous dilution of brilliant green into each tube prior to collecting a 10 cc. sample, gives a final dilution of 1:50,000 brilliant green in the milk. This dye greatly inhibits these contaminants, thereby increasing the efficiency of the test if such bacteria are present.—*M. W. Jennison.*

KNOWLES, R., DAS GUPTA, B. M. and BASU, B. C. **Studies in avian spirochaetosis. Parts I and II.** *Indian Med. Res. Memoirs No.* 22. 113 pp. 1932.

Dobell's modification of Levaditi's stain for spirochaetes in tissue leaves the organisms jet black against a colorless background with no black deposits or impregnation of fibrils. It is particularly recommended for insect tissue.

Put tissue slices no thicker than 1 mm. into a 1:9 formalin-saline mixture. Fix for at least 3 months, longer if possible. Transfer directly to abs. alcohol for 2–3 days. Pass down thru graded alcohols (90%, 75%, 50%, 1 day in each) to dist. water. Remove every trace of formalin by repeated changes of dist. water (at least 6 changes in 24 hr.). Place in 0.25–0.5% aq. $AgNO_3$ for 24 hrs. at 37°C. Darkness here is not essential but the proper temperature is important. A solution weaker than 0.25% will cause granules to develop in the tissue. The solution must be neutral.

Soak for 24 hr. or longer in numerous changes of dist. water in the incubator to wash out every trace of Ag. Develop in 0.5–1% hydroquinone or amidol, *not* in

pyrogallic acid-formalin. Make this solution by dissolving the hydroquinone in warm water; then drop the tissue in it. Leave for about 24 hr. Wash well with dist. water. Pass thru graded alcohols to xylene and paraffin; section; remove paraffin with xylene, and mount in balsam. Counterstains may be used.—*S. H. Hutner.*

SANDIFORD, B. R. **An improved method of staining for the demonstration of Neisseria in cellular exudates.** *J. Path. & Bact.*, **45**, 467–8. 1937.

The stain combination originally proposed by Fauth is recommended for smears with few diplococci but abundant confusing cellular debris. Proceed with the ordinary Gram stain up to the end of decolorization, then use the following Pappenheim stain: methyl green, 0.15 g.; pyronin, 0.5 g.; 95% alcohol, 5.0 cc.; glycerol, 20 cc.; make up to 100 cc. with 2% phenol. Leave on 2 min., wash briefly and blot. The stain keeps at least 2–3 months at room temp. Gram positive organisms are the usual purple black; nuclei, bluish-violet; cytoplasm, pale rose or violet.—*S. H. Hutner.*

STILLE, B. **Zytologische Untersuchungen an Bakterien mit Hilfe der Feulgenschen Nuclealreaktion.** *Arch. Microb.*, **8**, 125–48. 1937.

The optimal conditions of preliminary hydrolysis for Feulgen staining of bacteria and yeasts fixed in several common fluids were found to be 60 min. at 40° with *N* HCl. More severe hydrolysis results in the artefact of diffuse staining. The stainable material in bacteria occurs as granules which are not rudimentary spores, reserve materials, or plasmolysis bodies. The author believes that the constant presence of these granules in the cell and the apparent correlation of their number with the life cycle bespeak their nuclear nature.—*S. H. Hutner.*

WARD, A. R. and MYERS, C. E. **Influence of dead bacteria on microscopic counts of pasteurized milk.** *Amer. J. Pub. Health*, **27**, 899–905. 1937.

In the Breed method for the microscopic examination of bacteria in milk, those staining with methylene blue are regarded as alive. The authors' results justify the belief that insufficient numbers of dead bacteria remain visible after pasteurization to impair the usefulness of direct microscopic counts made on pasteurized milk.—*M. W. Jennison.*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stains approved since the last one listed in the July number of this journal.

STAINS CERTIFIED JUNE 1, 1937 TO NOV. 30, 1937*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Crystal violet	CC-9	88%	As histological, cytological and bacteriological stain	July 19, 1937
Crystal violet	NC-18	90%	As histological, cytological and bacteriological stain	July 21, 1937
Erythrosin	NEr-4	88%	As histological stain	July 26, 1937
Basic fuchsin	NF-30	83%	For general staining, the Feulgen reaction, and in Endo medium	Aug. 2, 1937
Methylene blue	LA-7	87%	For histology, bacteriology, and as constituent of blood stain	Oct. 4, 1937
Basic fuchsin	CF-16	88%	For general staining, the Feulgen reaction, and in Endo medium	Oct. 26, 1937
Methylene blue	CA-16	85%	For histology, bacteriology, and as constituent of blood stain	Oct. 26, 1937
Thionin	NT-5	86%	As histological stain and for frozen tissue	Nov. 8, 1937

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.